Growth of two species of Southern Ocean copepod in relation to their environment

Thesis

Growth of two species of Southern Ocean copepod in relation to their environment

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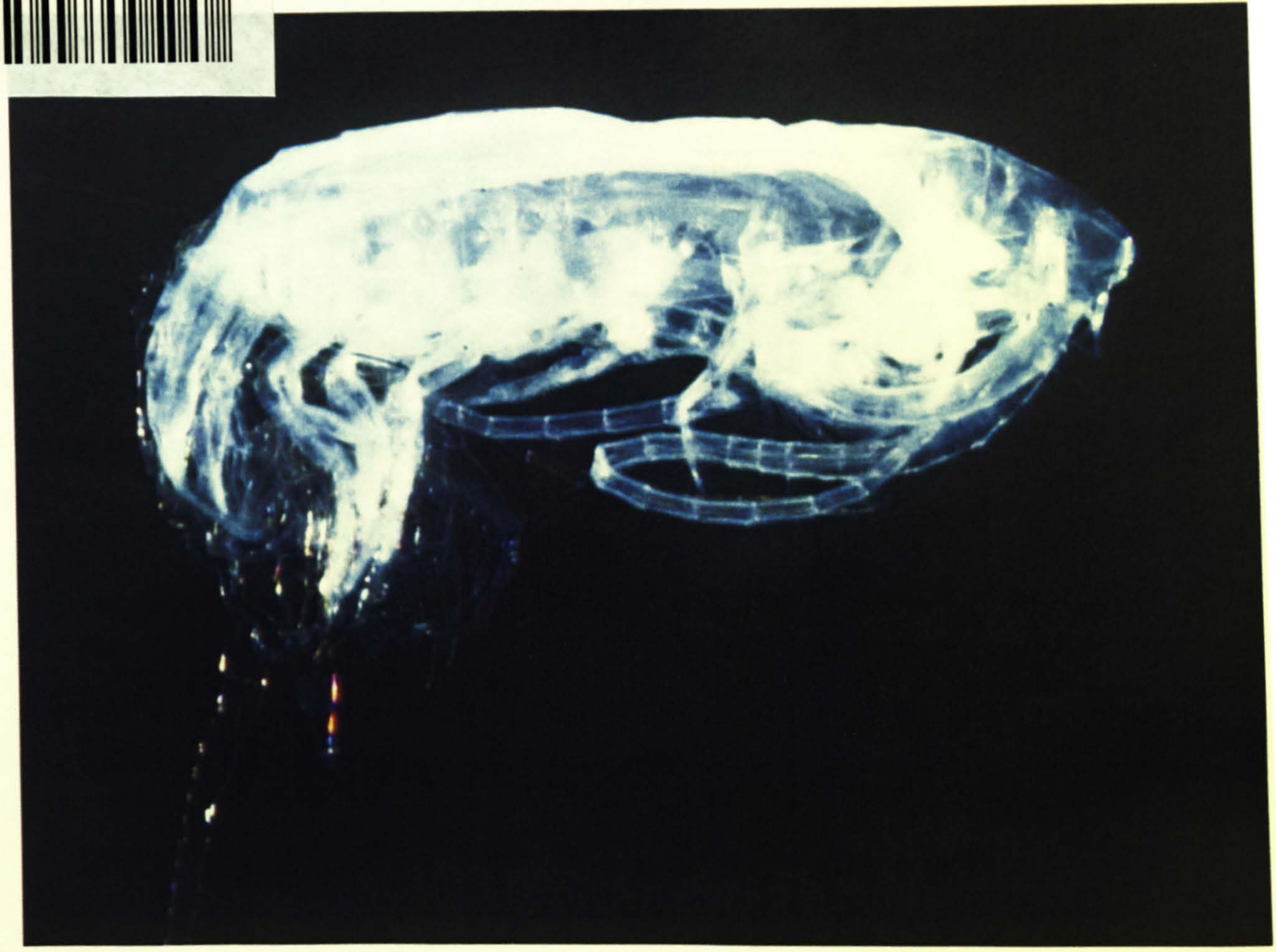

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This thesis is dedicated to the memory of Ron E. Shreeve.

Chapter 1 Introduction

Copepods are probably the most numerous metazoan sub-class on earth (Mauchline 1998). They are present in all water masses with the majority occurring in the marine environment. In the Oceans they comprise about 80% of the mesozooplankton biomass, as well as dominating numerically (Verity & Smetacek 1996). Copepods are the principle metozoan grazers in the oceans, and form one of the main links between primary production and commercially exploited pelagic fish (Turner 1984, Williams et al. 1994).

In order to appreciate the role of copepods in the energy flow in the marine ecosystem, it is essential to determine their rates of growth and production, and the factors which regulate these. Over the last few decades, considerable effort has been put into determining species-specific growth rates of copepods, and their contribution to community secondary production. These studies tend to be dominated by species from temperate, tropical or inshore habitats, and generally focus on factors controlling female egg production. Whilst measurements of egg production by adult females are most frequently used to represent growth for all developmental stages (McLaren & Leonard 1995, Hay 1995, Poulet et al. 1995), it has been suggested that this approach may considerably underestimate juvenile production (Peterson et al. 1991).

Far fewer studies have measured somatic growth of copepodite stages. This is despite the fact that growth and feeding rates of smaller copepods and early copepodite stages are much greater than those of adult females at the higher end of the size range for each species (Kiørboe & Sabatini 1995, Hirst & Sheader 1997). It is also becoming

increasingly apparent that population dynamics depends greatly on the survival and growth of the larval stages, which are more poorly known about than the adults.

In the Southern Ocean, copepods dominate the biomass (Voronina 1998) and are also believed to dominate secondary production by all zooplankton (Conover & Huntley 1991, Voronina 1998). However growth studies are dominated by krill, and a few egg production studies (Huntely & Escritor1991, Huntley & Lopez 1992, Kurbjewit 1993, Lopez et al. 1993 and Ward & Shreeve 1995) are the only examples of copepod growth. Somatic growth of Southern Ocean copepods has never been measured directly before.

This thesis sets out to measure the instantaneous growth rates of two key Southern Ocean species of copepod which occur commonly around the island of South Georgia, *Rhincalanus gigas* and *Calanoides acutus*, and to relate these measurements to key environmental variables.

This first chapter therefore deals with the location and historic interest in the island of South Georgia, and describes the physical environment typical of this area. It outlines the position of copepods in the food web, and describes the two key species chosen for this study. Finally the overall rationale of the project is explained.

1.1 Study location and physical characteristics

South Georgia is an Antarctic island, approximately 170 km long and about 35 km wide, that lies in the Atlantic sector of the Southern Ocean (Fig. 1.1). It forms part of a submarine ridge system, known as the Scotia Arc, which connects the Antarctic Peninsula to South America. The Scotia Arc encloses the Scotia Sea which is bounded to the west by the Drake Passage. The shelf region around South Georgia is generally less than 200 m deep and extends between 50 - 150 km from the coast; water depth then

increases rapidly to over 2000 m. Surface water temperatures range seasonally from less than 0°C in winter to more than 4°C in summer and there is considerable inter-annual variability in oceanographic conditions around the island (Whitehouse et al. 1996, Whitehouse et al. 2000). Distinctly colder years were identified by Deacon (1977), who linked them to variations in ice cover further south. More recently the duration and extent of the fast-ice around the South Orkneys in the southern Scotia Sea has been found to be negatively correlated to the surface water temperatures around South Georgia the following summer (Murphy et al. 1995, Whitehouse et al. 1996), so the cooler summers may be a result of basin-scale cooling caused by sea-ice extent and duration, as opposed to a shift in water mass distribution. The island lies in the northern Scotia Sea within the ice-free region of the Antarctic Circumpolar Current (ACC), well south of the Polar Front. This zone is largely oligotrophic and exemplifies the high nutrient and low chlorophyll (HNLC) environment typical of much of the Southern Ocean. However the South Georgia region does not typify these conditions, and can be extremely productive (Whitehouse et al. 1999, Atkinson et al. 2001).

1.1a Phytoplankton

During the austral summer, phytoplankton blooms are prevalent on the shelf area surrounding the island where chlorophyll *a* concentrations can occasionally exceed 20 mg m^{-3} (Whitehouse et al. 1996). Satellite-derived ocean colour measurements indicate that extensive surface phytoplankton blooms can develop around the island from November onwards and persist throughout the summer (Atkinson et al. 2001).

Primary production at high latitudes is typically of a highly seasonal nature and of a short duration (Clarke 1985, 1988), and the timing and magnitude of phytoplankton

blooms in the South Georgia region varies considerably (Atkinson et al. 2001). Further, primary production is also spatially very patchy. Surface water chlorophyll *a* and nutrient concentrations are patchy over scales ranging from a few, to hundreds of kilometres (Whitehouse et al. 1993).

Figure 1.1 South Georgia in relation to the Antarctic Peninsula, South America and the Scotia Arc. Four isobaths at depths of 200, 500, 1000 and 2000 m are marked in different colours for clarity. Figure supplied by B.A.S. Mapping and Geographical Information Centre using bathymetry reproduced from GEBCO, and made available through the GEBCO Digital Atlas published by the British Oceanographic Data Centre on behalf of the IOC and IHO.

Figure 1.1

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1.1b Zooplankton

Associated with the elevated phytoplankton production around the island, zooplankton biomass often reaches levels 4-5 times those more typical of the Southern Ocean (Mackintosh 1934, Ward et al. 1995, Atkinson et al. 1996). The distribution and abundance of zooplankton also exhibits great spatial and temporal variability. Inter-annual variations in abundance of krill (*Euphausia superba*), which do not originate around South Georgia, but are transported from further south, can be particularly marked. Brierley et al. (1997) have noted an inverse relationship between temperature and krill biomass. Their suggestion that krill are abundant in the southern part of the ACC and that increased abundance around South Georgia reflects a northwards extent of this colder water towards the island is in agreement with current ideas of krill transport across the Scotia Sea (Hofmann et al. 1998). In contrast, copepods may complete their entire life cycle around the island and the relative abundance of copepods and krill has been reported to describe a negative relationship (Atkinson et al. 1999). The relative importance of both physical and biological factors on copepod distribution were discussed by Atkinson et al. (1999). They found that, on a fine scale, krill had a direct effect on copepod abundance, either through predation or competition; on an inter-annual scale they suggested that variation in the abundance of copepods could be due to the physical environment, or the timing of the phytoplankton bloom.

1.2 Historical interest in the South Georgia region

The high biomass of zooplankton around South Georgia has been implicated in several major fisheries which have operated in this area (Everson 1984). The first of these was in the late 1700's and early 1800's when the Antarctic fur seal (*Arctocephalus*

gazella) was hunted to near extinction (Bonner 1984, Headland 1984). During the 20th century a fishery for baleen whales operated from the north of the island (Harmer 1931, Kemp & Bennet 1932). This ceased in 1965 as stocks declined (Moore et al. 1999). More recently fin-fish and krill fisheries have developed and are currently operating to the north of South Georgia (Everson 1984). This level of exploitation prompted early research into the key components of the food web, and their interactions in the ecosystem around South Georgia, with the objective of maintaining sustainable fisheries. The 'Discovery Expeditions' sampled intensively in the Southern Ocean in the period 1926 to 1951, initially examining the factors affecting the abundance and distribution of whales (Kemp et al. 1929). These authors found that the greatest whale numbers were found to the NE of the Island, and that their abundance varied on a seasonal and inter-annual basis. This variation appeared to be positively related to krill abundance, which in turn was related to an influx of colder water from the Weddell Sea which influenced the water conditions to the NE of the island (Marr 1962, Deacon 1977). More recent studies of inter-annual variability in the Scotia Sea between 1981 and 1984 have also shown great variation in the abundance of krill (Heywood et al. 1985, Priddle et al. 1988), with periodic (once or twice per decade) seasons of low krill abundance resulting in the breeding failure of land-based krill predators (Croxall et al. 1984).

Consequently much of the latter research work carried out on the zooplankton around the island focussed on krill, because the early assumption was that the energy flow in the Southern Ocean could be characterised by a simple linear food chain, leading from phytoplankton, through krill, to whales. However this concept has been revised in the light of the realisation that complex systems exist, exhibiting great spatial heterogeneity and numerous alternative pathways of energy transfer (Clarke 1985, Hempel 1985). An

important element in these alternative pathways are the copepods which frequently dominate the zooplankton biomass in the South Georgia area.

1.3 Copepods

Copepods dominate the zooplankton in the Atlantic sector of the Southern Ocean, accounting for about 60% of the biomass (Voronina et al. 1994) and at South Georgia accounting for between 41 and 98% of total zooplankton abundance (Pakhomov et al. 1997). Copepods are of prime importance in the marine ecosystem because many are herbivorous and form one of the main links between primary production and higher trophic levels. At South Georgia grazing impact by copepods on the primary production appears to be highly variable. Atkinson (1996) estimated that approximately < 5% of the total primary production was removed per day by copepods. Whilst estimates derived from Pakhomov et al. (1997) suggest that, in post-bloom conditions, large copepods consumed between 3 and 55% of daily primary production.

In turn copepods provide food for higher trophic levels. In the region of South Georgia copepods have been reported as being present in the diets of other copepods (Øresland & Ward 1993), fish larvae (North & Ward 1990), amphipods (Pakhomov & Perrissinitto 1996), krill (*Euphausia superba*) (Atkinson & Snÿder 1997), some species of birds (Croxall & Prince 1987), and baleen whales (Gaskin 1982).

Given the large temporal and spatial changes in the dominant zooplankton groups in the Southern Ocean, it has became apparent that to better understand the ecology in this area, we must look in more detail at these groups, their interactions and the effect that the physical environment has on their abundance, growth and development. Consequently more recent work has focussed on the larger biomass-dominant copepods which are

described below.

Two species of calanoid copepod were used for moulting and growth rate experiments in this study: *Calanoides acutus* and *Rhincalanus gigas* (Fig. 1.2). They were chosen because together they may comprise on average 37% of the total mesozooplankton biomass around South Georgia (Ward et al. 1995, Pakhomov et al. 1997). They are also large species, which facilitates quantitative capture of their early copepodite stages and allows accurate identification at sea with the minimum of handling. *Calanoides acutus* range from 1-2 mm long in stage CI to 6 mm in stage CVI adult females. *Rhincalanus gigas* is even larger with stage CI being approximately 2 mm long and stage CVI females reaching about 9 mm.

1.3a *Calanoides acutus*

Calanoides acutus was first described by Giesbrecht in 1902 from the collections of plankton samples made by the S.Y. Belgica. Mackintosh (1934) observed in Antarctic copepod assemblages that, 'numerically this is the most important species of all in the macroplankton.' This observation has been supported by the work of Hopkins (1971), Voronina (1970) and Pakhomov et al. (1997), who also found *C. acutus* to be one of the main contributors to zooplankton biomass in the Southern Ocean. It is because of their abundance that this species has been studied extensively. Initial work by Ottestad (1932) concentrated on its distribution and life cycle. Major events in the life cycle were documented by Vervoort (1965) and Andrews (1966). Ealey & Chittleborough (1956) were the first to suggest that this species commenced spawning as early as November, and they noted that females did not occur in their plankton samples between March and July.

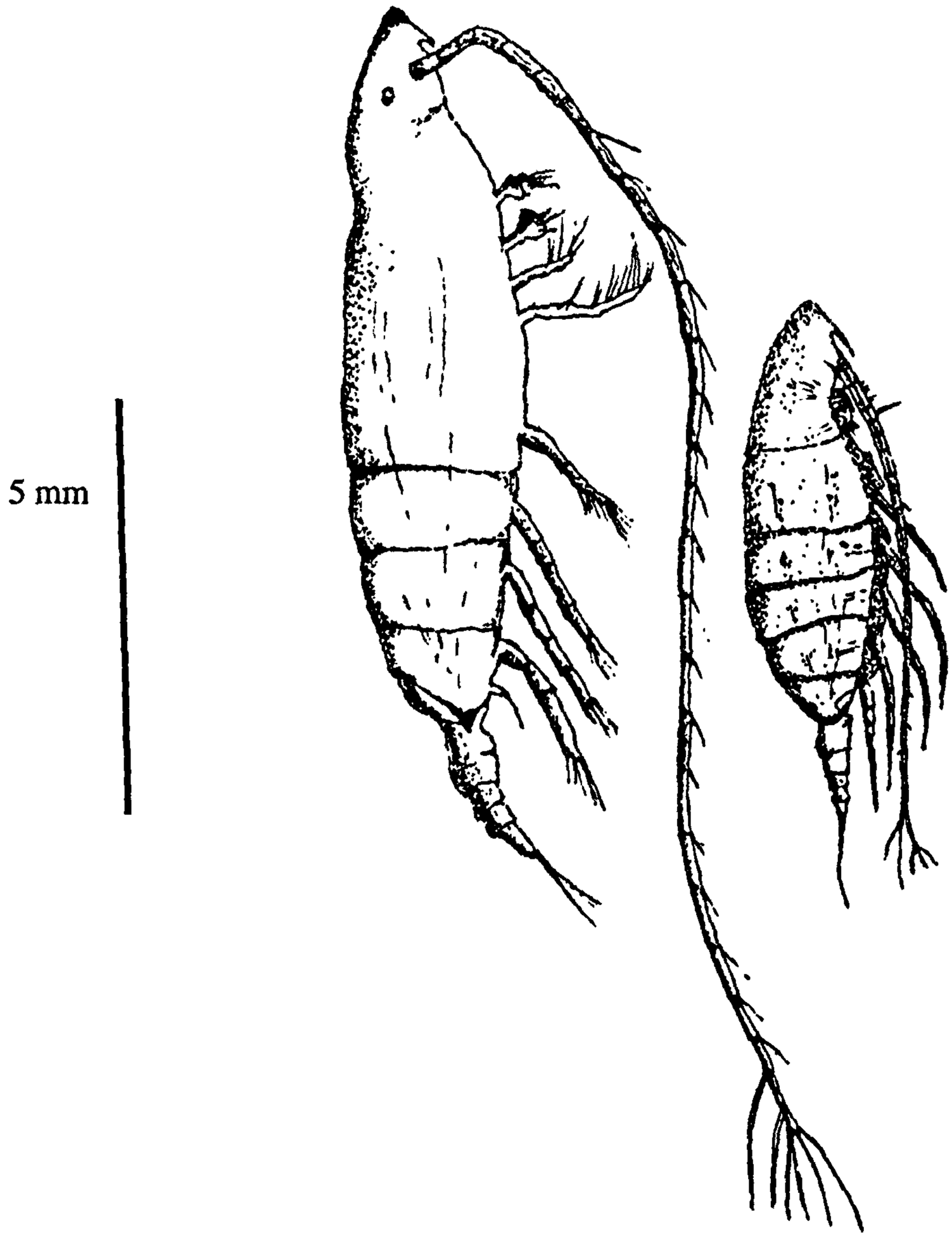


Figure 1.2 *Rhincalanus gigas* (left) and *Calanoides acutus* adult females.

During the 1990's studies began on more detailed aspects of life histories and physiology, such as abundance and distribution (Bathmann et al. 1993, Schnack-Schiel & Hagen 1995), diet and feeding (Schnack-Schiel et al. 1991, Atkinson et al. 1992, Hopkins et al. 1993, Pasternak et al. 1994), lipid storage (Graeve et al. 1994, Kattner et al. 1994, Pasternak et al. 1994, Hagen & Schnack-Schiel 1996) development rates (Atkinson 1991, Huntley & Escritor 1991, Lopez et al. 1993), and generation time (Atkinson et al. 1997). These studies have reached a consensus on most aspects of its biology in that it shows a circumpolar distribution, bounded to the north by the Polar Front and to the south by the Antarctic continent. *C. acutus* is primarily herbivorous and uses wax ester as a lipid store whilst undergoing diapause during winter. Its life cycle appears to be of a one year duration throughout much of its range, but this may be extended to two years at high latitudes.

However, there is still considerable uncertainty over the moulting and growth rates of *Calanoides acutus*, particularly of the younger copepodite stages. Huntley and co-workers (1994) studied the development and growth of *C. acutus* and approached the problem using methods based on the development of the stage frequency of a natural population over time. This method does not, however, allow a high resolution of the progression of the cohort because advection means that it is unlikely that the same population is sampled over time. Consequently robust estimates of stage-specific durations and growth rate in *C. acutus* have still not been established.

1.3b *Rhincalanus gigas*

The life history and ecology of *Rhincalanus gigas* is less well understood than that of *Calanoides acutus*. It is believed to have a similar life history to *C. acutus*

(Voronina 1970, Hopkins et al. 1993) but this is still controversial (Marin 1988, Atkinson 1991, Bathmann et al. 1993, Schnack-Schiel & Hagen 1994). Its life cycle was first described by Ommanney (1936), and details of its life cycle and physiology have been reported concurrently with those of *C. acutus* (see above). *R. gigas* is essentially a species of the Antarctic and Polar Frontal Zone, occurring at its greatest abundance in an area which is defined to the north by the Polar Front, and to the south by the 0°C isotherm. This species has been found at higher latitudes, although it would appear that these populations originate from a tongue of water that spills down into the Weddell Sea within the southerly moving deepwater of the east wind drift. In this area it is only a minor component constituting less than 15% of the copepod plankton (Ommanney1936). The life cycle of *R. gigas* again appears to be one year over much of its range, but may extend to two years in the higher latitudes (Marin 1988, Ward et al. 1997). *R. gigas* is thought to be primarily a suspension feeder, similar to *C. acutus* or slightly more omnivorous, and it also relies on the storage of wax ester to sustain it through its long winter diapause. Schnack et al. (1985) were the first to report rates of ingestion, assimilation and respiration for this species. Atkinson et al. (1992) calculated daily food rations and Ward & Shreeve (1995) reported egg production rates, but stage durations and growth rates remain poorly defined.

1.4 Project rationale

Studies to date have therefore still not fully resolved some of the fundamental issues in the life cycle of these key copepod species such as the timing of the onset of spawning, factors responsible for the flexible generation times and how variable the summer growth rates, body mass, stage durations and egg production rates are. We are

also still largely ignorant of how environmental factors affect these processes. Further studies on the stage-specific development of key species is therefore needed to help resolve these basic questions. The underlying problem with answering these questions is the difficulties that arise in the study of the Southern Ocean ecosystem. The remoteness of the area causes logistical constraints which generally only allow 'snap shots' of the ecosystem, of roughly one month duration at any one time and generally during the austral summer. Consequently many aspects of the life cycles have to be inferred, or rates assumed, for use in life cycle models and these are sometimes questionable. For example, growth rates have been derived by Conover and Huntley (1991) for *Rhincalanus gigas* and *Calanoides acutus* from measurements of ingestion, assimilation and respiration made by Schnack and co-workers (1985). Whilst until recently these were the only robust field estimates of rate processes available from the Southern Ocean, the application of the growth rates derived from them are questionable. Conover and Huntley (1991) derived growth rates that generally predict generation times well in excess of those that can reasonably be inferred from the stage frequency data for either species (Atkinson et al. 1997 and Ward et al.1997).

Given the temporal and spatial variability in both the biological and environmental parameters around South Georgia, and in a quest to further our understanding of it, the British Antarctic Survey carried out a multi-disciplinary research programme over a five year period. Research cruises took place during four consecutive austral summers between 1995/96 and 1999/2000 between mid December to mid January, a period which generally coincides with the peak of phytoplankton productivity (Whitehouse et al. 1993, 1996). A planned fifth cruise was cancelled due to the research

vessel being delayed in pack ice earlier in the season. The programme collected data on aspects of the physical and biological oceanography in an area to the north of the island of South Georgia which has a long history of fisheries-associated research. It therefore provided a good starting point for further research as we already have started to develop conceptual ideas of how the marine ecosystem in this area functions. These four cruises allowed the examination of variability in field estimates of growth and development of the younger copepodite stages of two major copepod species in relation to environmental variables, and these have been related to their rates of recruitment and abundance.

1. 5 Aims and layout of thesis

The aims of this study are

1. To determine development times of the younger copepodite stages of *Rhincalanus gigas* and *Calanoides acutus*, to help clarify the generation times of these two species.
2. To measure the range in carbon mass within each stage of the two key species, and the mass they attain upon moulting.
3. To measure mass specific growth rates for stages CI - CVI female of *Calanoides acutus* and *Rhincalanus gigas*. These are compared to global growth models that are documented in the literature.
4. To investigate the development, growth and abundance of the copepods in relation to key environmental variables, temperature and food, and to a potential key competitor/predator, krill (*Euphausia superba*).
5 To develop a conceptual model of how larger scale variability around South

Georgia may affect copepod growth rates, recruitment and overall abundance, and thus identify the major controls on copepod population dynamics in the South Georgia region.

In the next chapter three methods which are currently used to estimate moulting rates in copepods are compared in contemporaneous experiments. A temperate water species of copepod, *Calanus helgolandicus,* was used in this study to facilitate testing of the methods in the UK prior to the first Antarctic field season, and allowing the choice of the method most applicable for use in the Southern Ocean. Chapter 3 investigates the variability in the environmental parameters during the course of this study. In Chapters 4, 5 and 6, stage duration, elemental composition and mass specific growth rates for the copepodite stages of *Rhincalanus gigas* and *Calanoides acutus*, are investigated in relation to variation in environmental variables. Chapter 7 presents data on the stage frequency of the populations of *R. gigas* and *C. acutus,* which are related to their growth and development presented in earlier chapters. The population dynamics of these two species are then compared to the whole copepod assemblage. The final chapter is a Concluding Discussion, addressing copepods in relation to the Southern Ocean food web, as well as comparing their biomass and production with congeners in the northern hemisphere.

Chapter 2 Choice of experimental method for moulting rate studies

Data also presented in Shreeve RS, Ward P, Murray AWA (1998) Moulting rates of *Calanus helgolandicus*: an inter-comparison of experimental methods. J Exp Mar Biol Ecol. 224:145-154.

2.1 Introduction

The logistical constraints of working at sea at high latitudes have restricted the way in which estimates of generation times, life cycles, growth and development of copepods have been assessed. Most commonly, estimates of generation times and descriptions of the life cycles of Antarctic copepods have been based upon the study of the progression of a cohort over a period of time. In off-shore environments the samples used for these analyses have however generally been taken over a relatively short period each year, most often during the austral summer, and in order to complete a seasonal picture data sets have been compiled from a number of years. The analyses are further confounded by the fact that samples have been taken over a wide geographical range, and thus of necessity, data have been spatially and temporally averaged, (Huntley & Escritor 1991, Atkinson et al. 1997, Ward et al. 1997). Data derived in this way integrate the effects of a wide range of temperature and food environments and consequently data sets show a sometimes poor resolution of cohort structure and their progression (Ward et al. 1997). Nevertheless this approach has facilitated the general description of the life cycle of some of the biomass dominants such as *Rhincalanus gigas* (Ommanney 1936, Ward et al. 1997), *Calanoides acutus* (Andrews 1966, Atkinson et al. 1997) and *Calanus*

propinquus (Schnack-Schiel & Hagen 1995). However these data are unsuitable for describing accurately the rates of stage specific growth and development, so to address these it is necessary to use alternative methods to make instantaneous measurements of moulting rate. There are basically three experimental methods described in the literature to do this, and the first aim was to establish whether these methods gave similar estimates of stage duration when compared in contemporaneous experiments. The second aim was then to assess which was most appropriate for field work in the Southern Ocean. The first of these methods is the 'Heinle graph' approach (Heinle 1966), in which estimates of moulting rates are made based on changes in the stage frequency of a cultured population over time. The second method is to select stages individually, and the third to sieve fractionate sets of stages of a population. The cohorts produced from either method are subsequently incubated and by counting the number of individuals that have moulted, the stage duration can be calculated from the proportion of moulted to non moulted individuals.

Heinle (1966) originally raised animals in the laboratory in order to follow the development of a single cohort from egg to adult in a controlled environment. Food concentration, light regime, temperature and predation were known variables and handling of the animals was kept to a minimum. Sub-samples of the population were taken at regular intervals, preserved and staged. The time interval between 50% of individuals exsisting in successive stages is taken as equivalent to the stage duration. The second method, first described by Burkill and Kendall (1982), creates an artificial cohort by picking out a particular species stage from a plankton sample and then incubating these in the laboratory under controlled environmental conditions. At the end of the

incubation, the number of individuals which have moulted can be counted and expressed as a proportion of the total number incubated. The third method creates artificial sieved cohorts by size fractionation of a natural plankton assemblage followed again by incubation in the laboratory. Counts of a particular species stage are then made from sub-samples taken before and after incubation and the moulting rate is calculated by comparing the proportion of that stage in the samples at the start and end of the incubation (Tranter 1976, Kimmerer & McKinnon 1987).

The purpose of this chapter is to compare the stage duration estimates from the three main methods described above. By comparing rates estimated from contemporaneous experiments, an assessment could be made of the effects that handling and rearing of a population under artificial conditions had on moulting rate, and what effects intrinsic natural variability would have on the stage duration. The calanoid copepod *Calanus helgolandicus* was chosen as the study species as it fitted a number of criteria fundamental to this study. Firstly a species of copepod was needed that was related to those which the moulting rate experiments were to be carried out on in the Antarctic, as it was anticipated that they would react to incubation conditions in a similar manner. Secondly individuals were needed that were easy to collect from UK waters with a generation time that was relatively short. This would allow comparison of the stage durations of a manipulated natural population with a population reared from egg to adult in experimental conditions, within a reasonable time frame and without the complication of a diapausing stage. Thirdly a species was needed in which the copepodite stages were large enough to facilitate accurate microscope identification of live individuals easily and reliably. *Calanus helgolandicus* fitted all these criteria.

2.2 Materials and methods

Sample collection

Animals used in these experiments were obtained from either a natural or a laboratory-reared population. Natural plankton samples were collected on six occasions during July 1995 using a 50 cm diameter ring net equipped with a 200 µm mesh net and 1 l solid cod end. Samples were taken from an established station (L4) located at the entrance to Plymouth Sound (50°15'N, 4°13'W). Immediately upon capture samples were diluted into opaque buckets containing 10 l of sea-water at ambient temperature and transported back to the laboratory for treatment, a process which took less than 4 hrs. Sea-surface temperature and particulate organic carbon were measured weekly in the sea around station L4. On each occasion particulate organic carbon levels were in excess of 300 mg m^{-3} and sea surface temperature remained at around 15°C.

Laboratory reared population

The laboratory-reared population was initiated with eggs collected from egg production experiments in which adult female *Calanus helgolandicus,* which had been taken from L4 during June 1995, had been held in incubations for 1-2 days. Three tanks, each of 125 l volume, were inoculated with approximately 4,500 eggs each. Tanks were subjected to a 12 hour dark/light cycle at 15°C and stirred with a paddle at 10 revolutions per minute. Each tank was fed daily to excess (> 300 mg carbon m^{-3}) with one of three algal cultures: the flagellate *Isochrysis galbana,* the dinoflagellate *Prorocentrum micans* or the diatom *Thalassiosira weissflogii.*

The 'Heinle' method

Stage frequency of the tank-reared populations was monitored at two day intervals by gently mixing the tank contents and removing two 1 l sub-samples, which were immediately preserved and the numbers of each stage determined. A Generalized Linear Model (McCullagh & Nelder 1989), with binomial errors and logit link function was then fitted to these data using Genstat 5.3.2 (Payne et al. 1993). The logit model is expressed as:

$$\log\left(\frac{N_i}{\left(\sum_{j=1}^{13} N_j - N_i\right)}\right) = a_i + b_i t \qquad (1)$$

where N_i is the number of individuals in a given stage, i, at time t days, and N_j is the total number in all stages (eggs - CVI) in the sample at time t; a_i and b_i are parameters to be estimated by maximum likelihood. The time, $t_{50,i}$, for 50 % of copepods to moult from egg to the given stage, i, was then calculated using the following equation:

$$t_{50,i} = -\frac{a_i}{b_i} \qquad (2)$$

Sorted stage method

Sorted cohorts of the natural population were prepared by picking out the dominant stages under a dissection microscope. Individuals of each separated stage were then incubated in groups of 30 for 48 hours in 2.5 l jars containing filtered sea-water at 15°C. After 48 hours the animals were preserved in 4% formaldehyde in sea-water and the number of copepodites which had moulted to the next stage counted. Sorted cohorts from the laboratory-reared population were prepared by gently mixing the tank water to ensure an even distribution of individuals and then rapidly removing the first 60 animals. These were separated into stages and incubated as for the natural population. These experiments were set up on the same days as the stage-frequency measurements made on the laboratory population. Moulting rates from the sorted method were calculated using the equation:

$$MR_i = \frac{N_{f,i+1}}{N_{s,i}} \qquad (3)$$

where MR_i is the moulting rate of stage i, $N_{s,i}$ is the number of individuals at stage i at the start of the 48 hour incubation, and $N_{f,i+1}$ is the number of individuals which have moulted to stage $i+1$ by the end of the incubation. To calculate the moulting rate per day MR_i was then divided by the length of the incubation in days. Stage durations (days) were calculated as the reciprocal of the moulting rates. Confidence intervals (95%) were constructed from statistical tables (Neave, 1981) using charts to give confidence intervals for the sample fraction; these were then transformed to reciprocals to express them as a function of time.

Sieve-fractionating method

Sieve fractionation of the natural plankton sample involved initially passing the sample carefully through a 600 µm mesh sieve. Individuals that passed through this mesh were sieved again through a finer mesh of 200 µm and those retained were transferred to a bucket containing 10 l of filtered sea-water. All sieving was carried out at 15°C, under low illumination and the samples were kept constantly submerged. The final sieved fraction was then divided by gently agitating the sample and removing nine 1 l aliquots. Three 1 l initial sub-samples were preserved at the beginning of the experiment by adding a 4% formaldehyde in sea-water solution. A further six 1 l sub-samples were transferred to 2.5 l jars already containing 1.5 l of filtered sea-water and set up on an incubation wheel at 15°C under constant low illumination for 48 hours, after which the copepods were preserved in formaldehyde and all stages were counted. Moulting rates in this case were calculated using the equation described by Peterson et al. (1991):

$$MR_i = \frac{\sum_{j=i+1}^{6} P_{f,j} - \sum_{j=i+1}^{6} P_{s,j}}{P_{s,i}} \qquad (4)$$

where MR_i is the moulting rate of stage i, $P_{s,i}$ is the proportion of individuals at stage i at the start of the 48 hour incubation, $j=i+1$ and $P_{f,i+1}$ is the proportion of individuals which have moulted to stage $i+1$ by the end of the incubation. This estimate of MR_i is then divided by the duration of the incubation in days. Stage durations were calculated as the reciprocal of the moulting rates. Mean stage duration and standard deviation were calculated for each stage. Confidence intervals were constructed by multiplying the standard error of the mean by the value of Student's 't', taken from statistical tables (Neave, 1981) for n-1 degrees of freedom, where n is the number of replicate samples.

2.3 Results

Variability in stage duration

Individual variability in stage duration was assessed by making direct observations of the persistence of a stage in the laboratory-reared population. This showed that the time between first and last appearance of a particular stage ranged from two days in the case of CII to 15 days for CV (Table 2.1). Eggs used to inoculate these experiments were however, spawned within less than two days and very similar environmental conditions were experienced by copepodites within each tank. This therefore suggests that intrinsic individual variability accounts for a wide range of stage durations in *Calanus helgolandicus* copepodites regardless of the effects of external environmental factors. However, although some stages did persist in the laboratory reared population for up to 15 days, this accounted for less than two percent of individuals in each sub-sample, and in this case more than 95% of the copepodites had moved onto the next stage within a third of this time. This suggests that intrinsic affects are of low significance in instantaneous measurements of moulting rates.

Effect of diet on stage duration

Confidence intervals (95%) of mean durations of laboratory-reared, sorted, stages CI - CIV fed on each of the three micro-algal diets overlapped in 11 out of 12 of the comparisons made (Fig 2.1). Thus, diet type appeared to have no significant effect on stage duration in these experiments. Data collected for each individual diet were therefore pooled for all sorted cohorts, as were those from changes in stage frequencies of the laboratory-reared population over time.

Table 2.1 *Calanus helgolandicus*. Number of days that a particular copepodite stage persisted in the laboratory-reared population, in relation to algal diet. Persistence estimated from sub-samples taken from the tank populations every other day and estimating the stage frequency.

Copepodite Stage	*Thalassiosira weissflogii*	*Isochrysis galbana*	*Prorocentrum micans*
I	4	7	6
II	2	8	5
III	10	10	8
IV	10	14	10
V	11	15	8

Table 2.2 *Calanus helgolandicus*. Total numbers of individuals (by stage) used for each method, followed in parentheses by either the number of times that stage appeared in sub-samples of the tank population (*), or the number of experiments carried out (**). Durations not determined (ND)

Copepodite Stage	Stage frequency Tank *	Sorted Tank **	Sorted natural **	Sieved natural **
I	133 (9)	165 (8)	ND	628 (6)
II	150 (10)	79 (5)	59 (2)	1096 (6)
III	251 (13)	198 (9)	136 (4)	1127 (6)
IV	174 (19)	270 (9)	94 (3)	543 (6)
V	338 (18)	ND	57 (3)	48 (1)

Effect of incubation in filtered sea-water

No significant differences were found between estimates of stage duration made by the Heinle method compared to those estimated by the sorted method on the laboratory reared population (Fig. 2.2). This suggests that the absence of food that copepods experienced during the period of incubation in the sorted method did not have a significant effect on moulting rates.

Comparison of methods

Mean estimates of stage durations and confidence intervals (95%) derived from the four experiments are shown in Figure 2.2 and the total number of animals used to estimate stage durations for each stage and method are shown in Table 2.2. Of the 24 comparisons made, 20 showed confidence intervals which overlapped, or mean values which fell within these intervals. There were no significant differences between methods and no one method gave stage duration estimates which were systematically higher or lower than another. Stage CIII was numerically dominant in each method tested, and gave the tightest confidence intervals, with all estimates of mean stage duration being within one day. Stage duration for CI estimated from the Heinle method was slightly shorter than that obtained from the sorted laboratory or natural sieved experiments. Estimates of stage duration for laboratory-reared CII using both methods gave mean values of up to 1.5 days shorter than for the natural population. For stage CV the Heinle method gave longer durations than for the natural population.

Figure 2.1 Mean stage duration and confidence intervals (95%) for *Calanus helgolandicus* stages CI - CIV, estimated from the sorted method performed on three tank reared populations fed on different micro-algal diets; (▲) *Thalassiosira weissfloggii*, (◇) *Isochrysis galbana* and (◆) *Prorocentrum micans*.

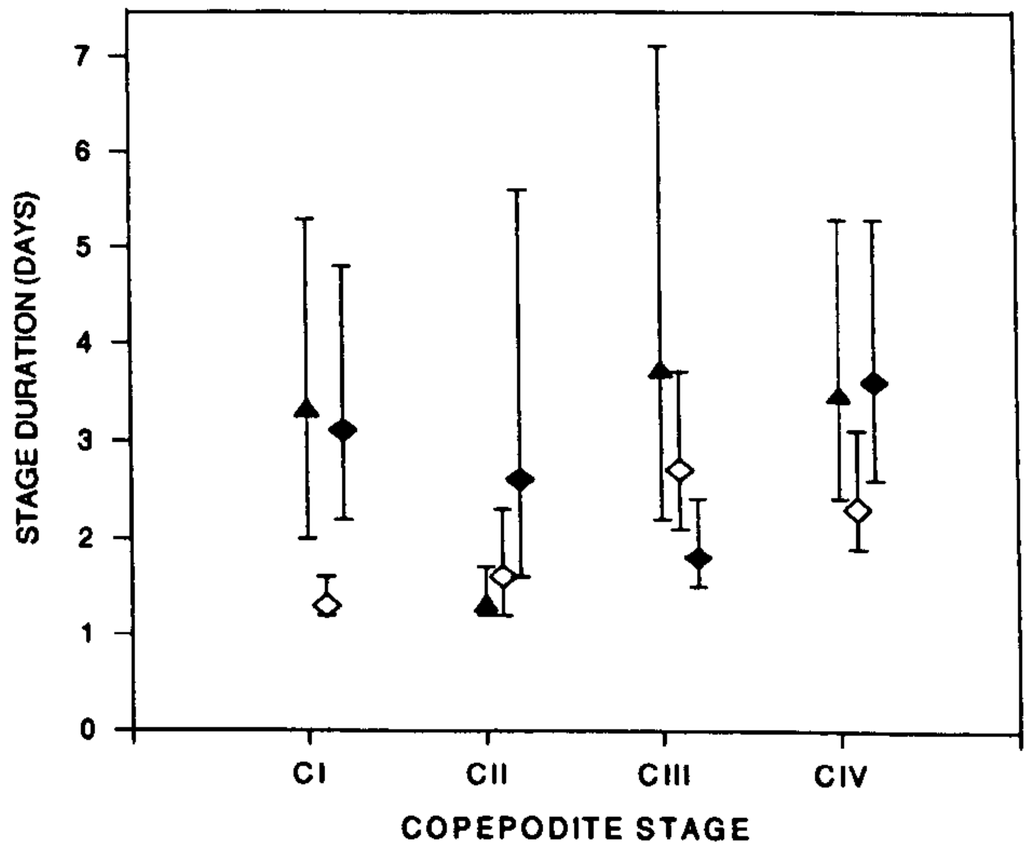
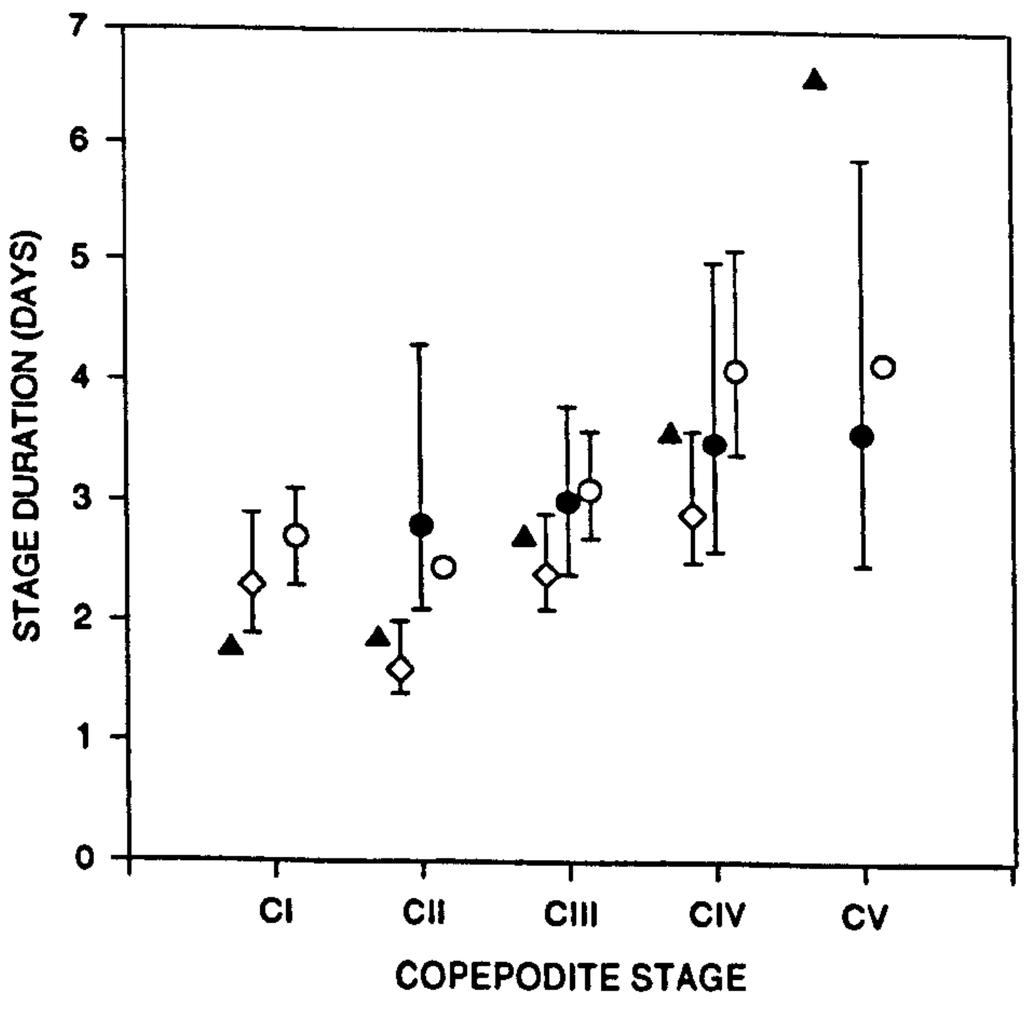


Figure 2.2 Mean stage duration and confidence intervals (95%) of *Calanus helgolandicus* estimated from; the 'Heinle' method (▲), the sorted method on the laboratory reared population representing a mean of all three diets (◇), the sorted method on a natural population (●), and the sieved method on a natural population (○).



Handling effects on the moulting rates of copepods were investigated by comparing rates from methods which involved different amounts of handling. Firstly the stage durations estimated by following the stage frequencies of the laboratory-reared population over time, (requiring the least amount of manipulation) were compared to those from sorting the same population (requiring some manipulation). Estimates for the stage duration of CI - CIV for the sorted method have confidence intervals with a spread of less than one day either side of the mean (Fig. 2.2). Stage durations for CII - CIV estimated from the Heinle method fall within these confidence intervals, indicating that there is no significant difference between the estimates made for these stages using these two methods. A further investigation of potential handling effects was made by the comparison of stage durations estimated from the sieved method, (where maximum handling of the individuals occurs) to estimates made by sorting the natural population. Differences between mean stage durations were again less than one day for stages CII - V, confidence intervals (95%) again overlapped and hence showed no significant differences. This shows that although the process of sieving has been criticised for damaging the copepods to a point where they can no longer moult (Miller & Nielsen 1988), in these experiments there were no detectable differences at least for stages CIII and CIV.

The effect that environmental parameters had on stage duration was investigated by comparing the durations estimated using the sorted method performed either on the laboratory-reared population or on the natural plankton samples. Differences in mean stage duration between the populations for CIII and CIV were less than one day (Fig. 2.2). Although tank-reared copepods generally gave shorter durations they were not

significantly different, so it suggests that the conditions under which the laboratory population was reared did not affect stage durations.

2.4 Discussion

The main questions addressed here were, first whether handling of the individuals would affect moulting rates significantly, and second, whether the rates estimated in the laboratory-reared population differed significantly from those of a natural population.

Handling effects

It has been suggested that handling of the copepods may either cause moulting bursts during the initial stages of the experiment and so produce an artificially elevated moulting rate (Miller et al. 1984). Alternatively handling may damage the animals and leave them unable to moult so causing an artificially lowered moulting rate (Miller & Nielsen 1988). Handling effects were investigated by comparing results from the Heinle method on the laboratory-reared population, a method which requires the minimal amount of handling, to the sorted method conducted on the same population. Similar durations were estimated by both, suggesting that sorting in these experiments did not significantly effect moulting rates either by causing moulting bursts or by damage. Similar results were found by Runge et al. (1985) who monitored changes in stage frequency of a natural population of *Calanus* spp. over time whilst simultaneous measurements of moulting rates of a sorted cohort were made on-board ship. The latter accurately predicted changes in the natural cohort structure. However, for such comparisons to be valid, it is necessary for the natural population to show near synchronous cohort development (Miller & Tande 1993). Peterson and Hutchings (1995)

also made an assessment of the effects of handling by comparing moulting rates estimated using the sorted and sieved methods on a natural population of *C. agulhensis*. They found no significant differences between rates estimated by these two methods, although both require a certain amount of handling.

Laboratory versus natural population

Direct comparison made between the laboratory-reared and natural populations using the sorted method showed no significant differences between the estimates of moulting rate made from these two populations. Peterson and Hutchings (1995) suggested that moulting rates of *Calanus agulhensis* in a natural population never reached those achieved by laboratory-reared populations due to food limitation. In our experiments food levels in the laboratory and in the sea-water around station L*4* remained at levels in excess of 300 mg C m^{-3}, suggesting that estimates of moulting rates from laboratory-reared populations can reflect those of the natural population providing food concentrations are similar. Comparisons of the three mono-cultured micro-algal diets also suggest that moulting rate was unaffected by the nature of the diet.

Although there are no reported ranges of stage durations for this species, the durations reported by Thompson (1982) for *Calanus* sp. (probably *C. helgolandicus*, Corkett et al. 1986), were within the ranges reported in this study at a similar temperature.

Age within stage

The age within a stage that copepods are in, prior to the initiation of moulting rate experiments, has been suggested as a potential source of error by Miller et al. (1984).

They suggest that if moulting is associated with some environmental cue, such as a diel or tidal cycle, there might not be an even spread of age within a stage. This could affect the moulting rates if the incubation period is less than the natural moulting cycle. However, the 'Heinle' method, which effectively looks at the population over its entire life cycle, helps to smooth any effects of pulse moulting. As stage durations derived by this method compared well with those derived from the other methods, this data suggests that no significant errors have been introduced by a non-uniform age within stage.

Application of methods

The different methods compared here did not appear to affect the estimates of stage duration in any significant or consistent way. Therefore, we can make an assessment of the most practical and applicable method to be used at high latitudes. The Heinle method is not appropriate as it relies on a field season which extends as long as the generation time of the copepods under study. This is not possible for ship-based work on a population of copepods which have a generation time of approximately one year.

The sieved method relies on high numbers of a particular species stage being present in the plankton sample at the initiation of the experiments. To control the mortality rate it also requires that any potential predators are separated from the copepodite stage of interest. Plankton hauls in the Southern Ocean rarely conform to these two criteria, therefore making this method unsuitable for use there.

The sorted method appears to be most suitable for use in the Southern Ocean where the main problems are a short field season, high advection rates and prolonged recruitment. This method allows an instantaneous measure of the moulting rate which is directly linked to the past feeding and temperature history of the animal. A typical visit

to a station will yield about 200 individuals of a particular species and stage and allow an accurate estimate of the stage duration.

The following chapter sets out the sampling procedures and characterises the prevailing environmental factors observed during this survey.

Chapter 3 Characterisation of environmental variables

3.1 Introduction

Instantaneous measurements of the stage duration (SD) and mass specific growth rate (g) of copepods made during this survey will be influenced by past environmental conditions. It is well documented that temperature and food availability can significantly affect growth rates in zooplankton both within and between species (Clarke & Peck 1991, Huntley & Lopez 1992). In laboratory experiments on calanoid copepods, stage duration was demonstrated to be longer in individuals incubated at lower temperatures (Vidal 1980b, Thompson 1982) and because g was found to be the same at all temperatures (Vidal 1980b), there was a greater increment in weight realised in each stage in copepods growing at lower temperatures. Vidal (1980a) also demonstrated that environmental variables may affect stage development in different ways. He showed that the dry mass of early stages was relatively unaffected by differences in either temperature or food concentration, but that the dry mass of the later stages increased hyperbolically with increasing food concentration and was inversely related to temperature.

Therefore, to place measurements of growth and development from this study into context, it is necessary to first measure and describe environmental variables at each sampling site. As instantaneous measurements of SD and g from natural copepod populations are being related to variable field environmental factors, a number of ways of expressing the temperature and food environment will be included. In this way it is hoped that the most important predictors of copepod growth and development in the South Georgia ecosystem can be determined. Temperature was expressed as both the

mean temperature in the top 60 m and the minimum temperature in the top 200 m. The former represents the depth range in the water column within which the majority of copepods species analysed in this study reside during the summer (Atkinson et al. 1992, Ward et al. 1995), whilst minimum temperature in the top 200 m gave an indication of the temperature of the water column within the mixed layer depth during the previous winter. The food environment was characterised by chlorophyll *a* concentrations which serves as an indication of phytoplankton abundance. Nutrient depletion was also considered as a proxy for the extent of the recent (days to weeks) phytoplankton productivity. Silicate was specifically chosen because the products of its biological use are re-mineralised far more slowly in colder water then any other commonly measured nutrient such as nitrate or phosphate, and generally does not occur within a season (Kamatani & Riley 1980, Nelson & Gordon 1982). Phytoplankton growth around South Georgia is dominated by diatoms which are reliant on silicate, therefore the amount of silicate depletion in the upper water column may be used as a proxy for phytoplankton productivity in the recent (days to weeks) history of the water column.

The Antarctic krill (*Euphausia superba*) has been included as an 'environmental' factor, as a high biomass is sometimes present around the island (Voronina et al. 1994, Voronina 1998). Krill may either directly predate the copepods or be a significant competitor for food (Atkinson et al. 1999). For example, in laboratory experiments, Granéli et al. (1993) found that krill rapidly cleared the phytoplankton biomass and that the remaining community became flagellate dominated. They observed a similar scenario *in situ* in the Scotia-Weddell Sea. Here they reported that a krill swarm grazed down a phytoplankton bloom in a few hours, leaving behind a flagellate dominated community. This high grazing pressure could potentially alter the local feeding environment for the

copepods for a significant period after the krill move out of the area.

South Georgia lies within the Antarctic Circumpolar Current (ACC), as described in Chapter 1. Within the ACC there are cores of water travelling at a higher velocity than neighbouring water. These currents are associated with thermohaline fronts. The Southern ACC Front (SACCF) has been identified as one of three major fronts in the ACC (Orsi et al. 1995). Lying between the Polar Front (PF) and the Southern Boundary (SB) of the ACC, the SACCF crosses the central Scotia Sea and flows around South Georgia before retroflecting north of the island (Orsi et al. 1995) (Fig. 3.1). The position of the SB of the ACC has been subject to much debate, and it is the waters between the SACCF and the SB that are important for oceanic transport to South Georgia (Hoffmann et al. 1998). Water from south of the SACCF may be colder, silicate replete and carry krill from the Antarctic Peninsula region to the area of South Georgia. The physical properties alone may account for any differences observed in the moulting, growth and population structure of the copepods. With this in mind, the water mass was first analysed to see if the stations sampled during this study always lay to one side of the SACCF.

In this chapter general patterns found in the environmental data are presented, and the relationships that exist between them. Much of these data were collected by colleagues, therefore only general descriptions of the sampling methods and the way in which the data was treated for the purpose of this study are covered here. More detailed descriptions of methods used by colleagues are referenced in each section.

3.2 Materials and methods

Study area

The main area of study was to the north of South Georgia, within two mesoscale areas which straddled the shelf. These are referred to as the Western Mesoscale Box (WMB) and Eastern Mesoscale Box (EMB) (Fig. 3.2). These two areas have been shown to display differences in their physical and biological characteristics (Marr 1962, Deacon 1977), which were outlined in Chapter 1. For each box, eight 80 km transect pairs were run perpendicular to the shelf break to undertake acoustic surveys (Fig. 3.2). Transects were surveyed starting from the south-west corner and then progressing north-eastwards, against the prevailing water currents. One transect pair was completed during each period between dawn and dusk. Each day, following the completion of the acoustic survey, the ship returned along the transect just completed, and stations 25 km from each end were sampled. This ensured that one on-shelf and one off-shelf station was sampled during each period of darkness, (in this study the term on-shelf refers to water depths of < 500 m, and off-shelf > 500 m). Station based work involved the deployment of zooplankton nets, CTDs and water bottle rosettes. Each element studied is described in sections below, and the results used to determine which environmental factors best predict the variability in copepod growth and abundance.

Physical environment

The physical properties of the water column were characterised at each station using a CTD with a rosette of water bottles. Details of the temperature and salinity measurements and their calibration can be found in Brandon et al. (1999), so just the

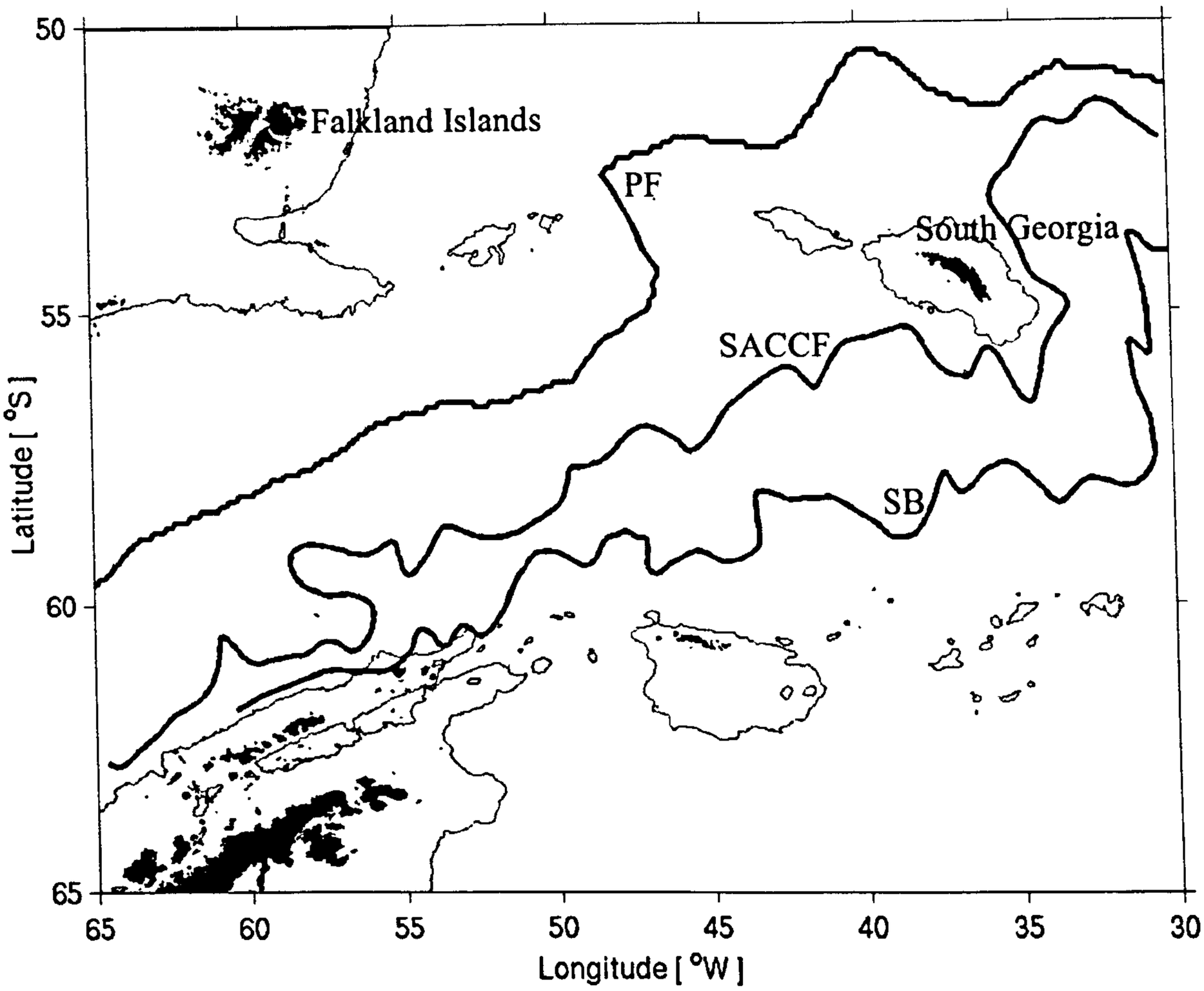


Figure 3.1. Position of fronts within the Antarctic Circumpolar Current (ACC) showing, Polar Front (PF), Southern ACC Front (SACCF) and Sounthern Boundary (SB) of the ACC, in relation to South Georgia. Dark shaded areas are land masses, grey shaded area water <1000m. PF after Moore et al. (1999),SACCF & SB after Thorpe (2001) ACC defined as all water flowing through Drake Passage.

essentials are covered here.

One on-shelf and one off-shelf CTD cast was made every day, the on-shelf being made to near bottom and the off-shelf to 1000 m. Temperature and salinity were recorded at 2 m intervals. Water samples were taken at standard depths of 6, 20, 40 and 60 m in order to calibrate the salinity sensor and to characterise the nutrient environment, chlorophyll *a* and phytoplankton assemblage. Data from these samples were integrated over the top 60 m of the water column, where the copepods used in this study generally reside during this time of year (Atkinson et al. 1992, Ward et al. 1995).

Nutrients were analysed by filtering water samples through a mixed ester membrane (Whatman WME, pore size 0.45μm) and the filtrate analysed colorimetrically for dissolved silicate ($Si(OH)_4$-Si) using a Technicon segmented flow analyser according to the method of Whitehouse and Woodley (1987). A further sub-sample of water was filtered through a glass fibre filter (Whatman GF/F, pore size 0.7μm), and the retained particulate material was analysed for chlorophyll *a* and phæopigments according to the method of Parsons et al. (1984). The GF/F filters were extracted in 90% aqueous acetone, and the fluorescence of the extract measured before and after acidification using a Sequoia Turner 112 fluorometer which had been calibrated with a commercially prepared chlorophyll *a* standard (Sigma Chemical Company, St Louis, MO, USA).

Figure 3.2 Study area showing the 500 m isobath, lines representing transect pairs, WMB and EMB, western and eastern mescoscale boxes respectively. Closed circles show station positions numbered in the order they were surveyed. 2, 3, 6 and 7 are off-shelf stations and 1,4,5 and 8 are on-shelf stations.

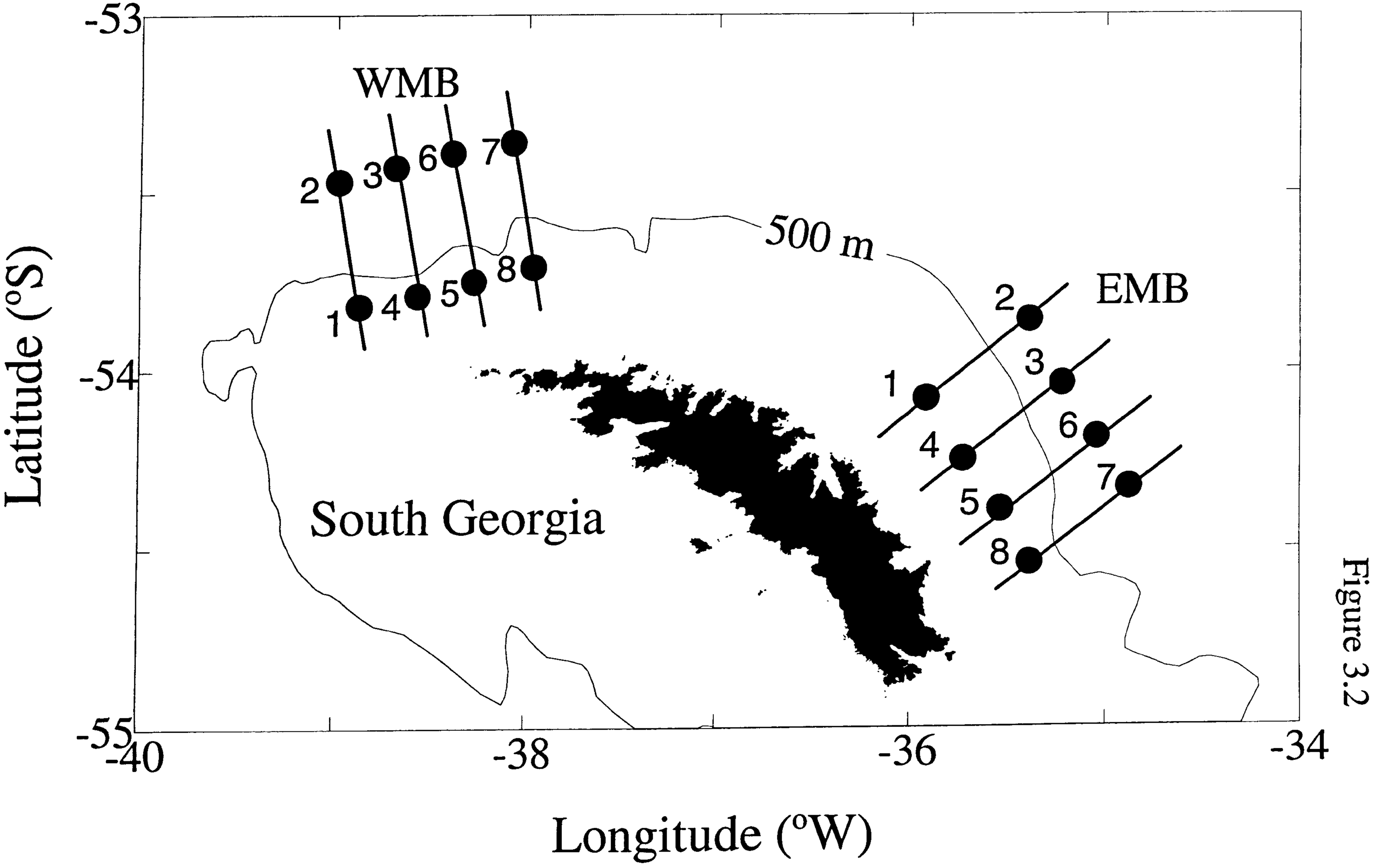

-53
-54
-55
-40
-38
-36
-34
Latitude (°S)
Longitude (°W)
WMB
1
2
3
4
5
6
7
8
EMB
1
2
3
4
5
6
7
8
500 m
South Georgia

Figure 3.2

The diatom and dinoflagellate fractions of the microplankton were defined at two on-shelf and two off-shelf stations in each box using HPLC analysis. Microplankton was sampled by filtration of seawater collected at the 30 m depth horizon. This was size fractionated onto ashed glass fibre membranes (Whatman grade GF/F, nominal retention 0.7 μm) and nylon membranes (nominal retention 20 μm) arranged in series with a 200 μm nylon mesh prefilter. These were then extracted in 1 ml of 90% acetone, using an ultrasonic cell disrupter. The acetone solution was centrifuged and the supernatant analysed by HPLC using the method developed from that of Mantoura & Llewellyn (1983). The carotenoid algal markers were identified by comparison of retention times with algae with known pigment profiles (Wright et al. 1991) grown from cultures supplied by the Plymouth Marine Laboratory.

Zooplankton net design and sample collection

Zooplankton were sampled at each station using a motion compensated Bongo net (Fig. 3.3). Copepods which had been caught with the old style of vertically hauled zooplankton net during previous field seasons often showed signs of physical damage. The old style of net was attached to a wire which was rigged directly to the winch. Wire tension would alter with the sea swell, causing a jerky recovery of the net and damage to the zooplankton. This was problematic as we needed individuals in as good a condition as possible, as damaged individuals may not moult successfully (Miller et al. 1984).

An alternative means of catching sufficient undamaged zooplankton was therefore required. The motion compensated Bongo net was therefore designed with this in mind. The Bongo frame consists of two mouth-rings each of 61 cm diameter, from which nets are held rigidly within a frame and a 5-litre non-filtering cod-end attached to the bottom

of each fitted with a tap at the base to facilitate retrieval of the catch. Wire from a winch onboard ship passes down through the Bongo frame to connect with a drum of wire tensioned by springs located half way down the net frame (Fig. 3.3) While at a constant speed, approximately half of the wire is unwound from the net drum. As tension is increased by heave at the gantry head wire is pulled off the net drum until the tension balances the new requirement or the motion reverses. Similarly when tension decreases wire is wound in, thus maintaining the speed of movement through the water.

To reduce damage to antennae and setae during collection of live copepods for experimental work, nets should be preferably of a fine-mesh relative to the size of the individual (Sameoto et al. 2000). Thus Bongo nets were routinely equipped with 100 and 200 μm mesh nets and were deployed to a depth of 200 m, or near bottom if bathymetry restricted. They were hauled to the surface at between 10 - 13 m min^{-1}, so that the sample was in the cod end for no longer than 20 mins. Once onboard, samples were diluted in approximately 5 l of ambient surface sea water in an opaque bucket. Copepods were then sorted for experimental work and the residue preserved in 4% formaldehyde in sea water for laboratory analysis in the UK. All copepod taxa in the preserved samples were enumerated under a dissection microscope and copepodite stage frequency determined for the dominant copepods. Where necessary sub-sampling took place using a Folsom splitter. Generally one fraction was used to enumerate the large copepods and another, smaller fraction, to enumerate smaller copepods. This facilitated the counting of approximately 400 individuals of the dominant copepod species.

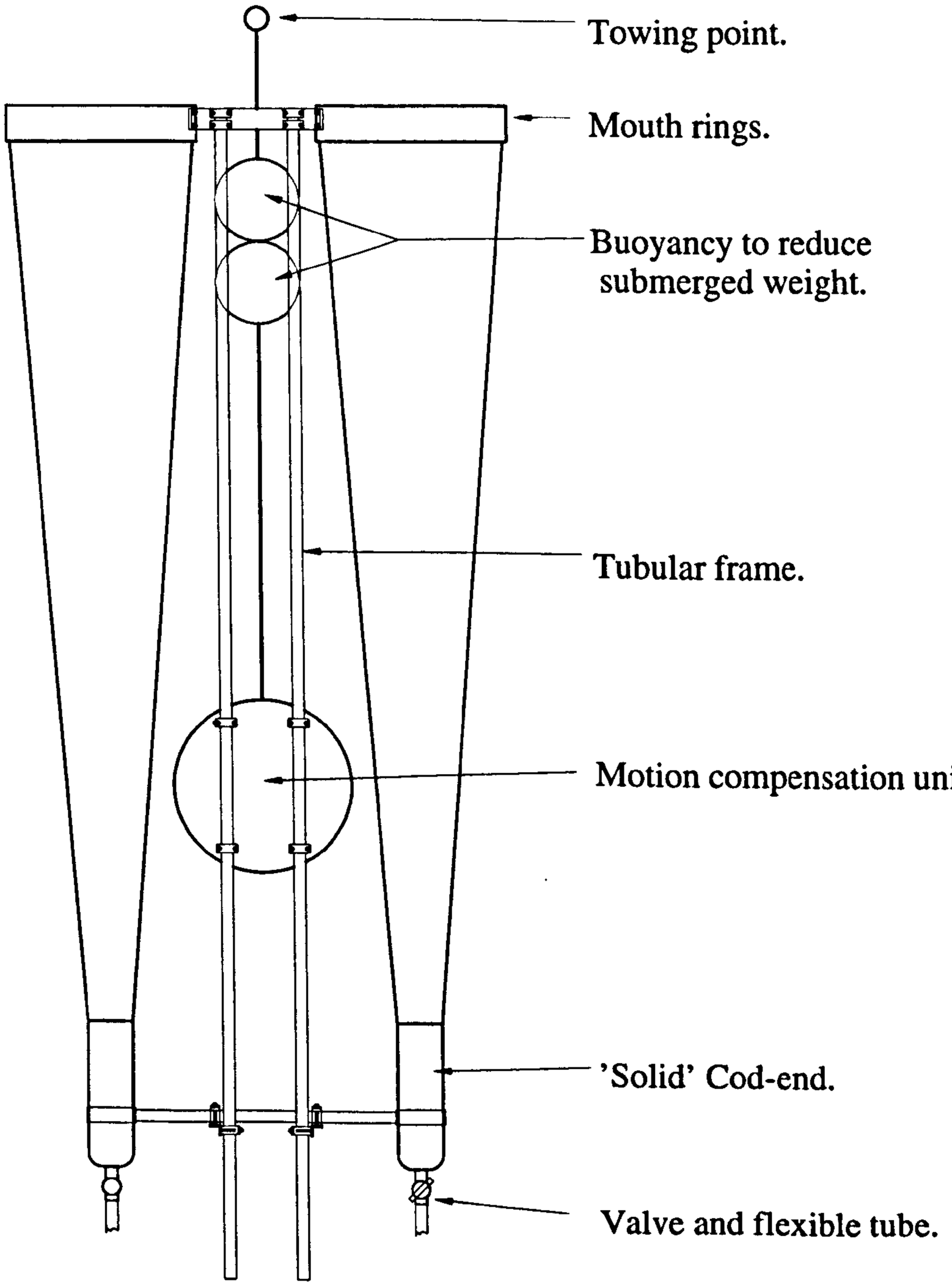


Figure 3.3 Schematic diagram of the motion compensated Bongo net.

Acoustic surveys

Acoustic surveys were conducted to assess the abundance of Antarctic krill (*Euphausia superba*). Full details of the acoustic survey, target identification and data processing are in Brierley et al. (1997, 1999), so only the essential survey design is covered here. Acoustic data were collected along the transect lines using an EK500 echo sounder, linked to hull-mounted 38 and 120 kHz split beam transducers. All acoustic surveys were conducted during the hours of daylight to avoid possible bias due to diel vertical migration (Demer & Hewitt 1995), or a change in target orientation (Everson 1982, 1983). Transects were run alternately in on- and off-shelf directions at a nominal speed of 10 knots, with the starting point alternated on a daily basis. Transects were surveyed sequentially from west to east, against the prevailing current flow (cf Deacon 1937), in an attempt to avoid 'aliasing' flow-mediated horizontal krill transport (MacLennon & Simmonds 1992). For the purposes of this study, to compare krill biomass with copepod abundance, krill biomass estimates are expressed as g wet mass (WM) m^{-2} integrated over the top 250 m and represent a mean density calculated 5 km either side of the station position on each transect.

3.3 Results and Discussion

Water temperature

Over the four summer surveys there was no evidence from the water mass sampled to suggest that the water was from anywhere but north of the SACCF. Analysis of θ max at depths greater than 500 m showed that temperature was above 1.8°C, a criterion defined by Orsi et al. (1995) as a property indicator of the SACCF.

Mean water temperature (0 - 60 m) measured across all stations during the four

cruises ranged from 1.24 to 3.32°C. General patterns in water temperature can be seen (Fig. 3.4). A comparison between years shows that the water temperatures found around the island during the 1997/1998 season were significantly colder than other years by 0.67 °C (Fig. 3.5a). Over the whole study period the mean water temperature (0 - 60 m) in the WMB was significantly warmer than the EMB by 0.55 °C (Fig. 3.5b). In the EMB warmer water occurred on-shelf (Fig. 3.5c), but no significant difference was found between on- and off-shelf locations in the WMB. Although differences were found between areas and years, there were no significant differences in the mean water temperature with regard to station location.

Silicate concentrations

Silicate concentrations integrated over the top 60 m of the water column ranged from 0.024 - 2.04 mol m^{-2}. General patterns in silicate concentration are evident between areas and years (Fig. 3.6). On a year to year analysis, greater depletion occurred during the 1998/99 and 1996/97 seasons (Fig. 3.7a) and was greater in the WMB compared to the EMB (Fig. 3.7b). Within boxes significant depletion occurred at the off-shelf site in the WMB (Fig. 3.7c) although there was no on-/off-shelf differences apparent in the EMB.

Figure 3.4 Mean water temperature (°C) (0 - 60 m) for all stations studied in all years.

○ 1.3 - 1.5, ◯ 1.51 - 2, ◯ 2.01 - 2.5, ◯ 2.51 - 3, ◯ 3.01 - 3.4

Shaded area shows the land mass of South Georgia, contour line shows the 500 m isobath. Some station data missing due to bad weather.

Figure 3.4

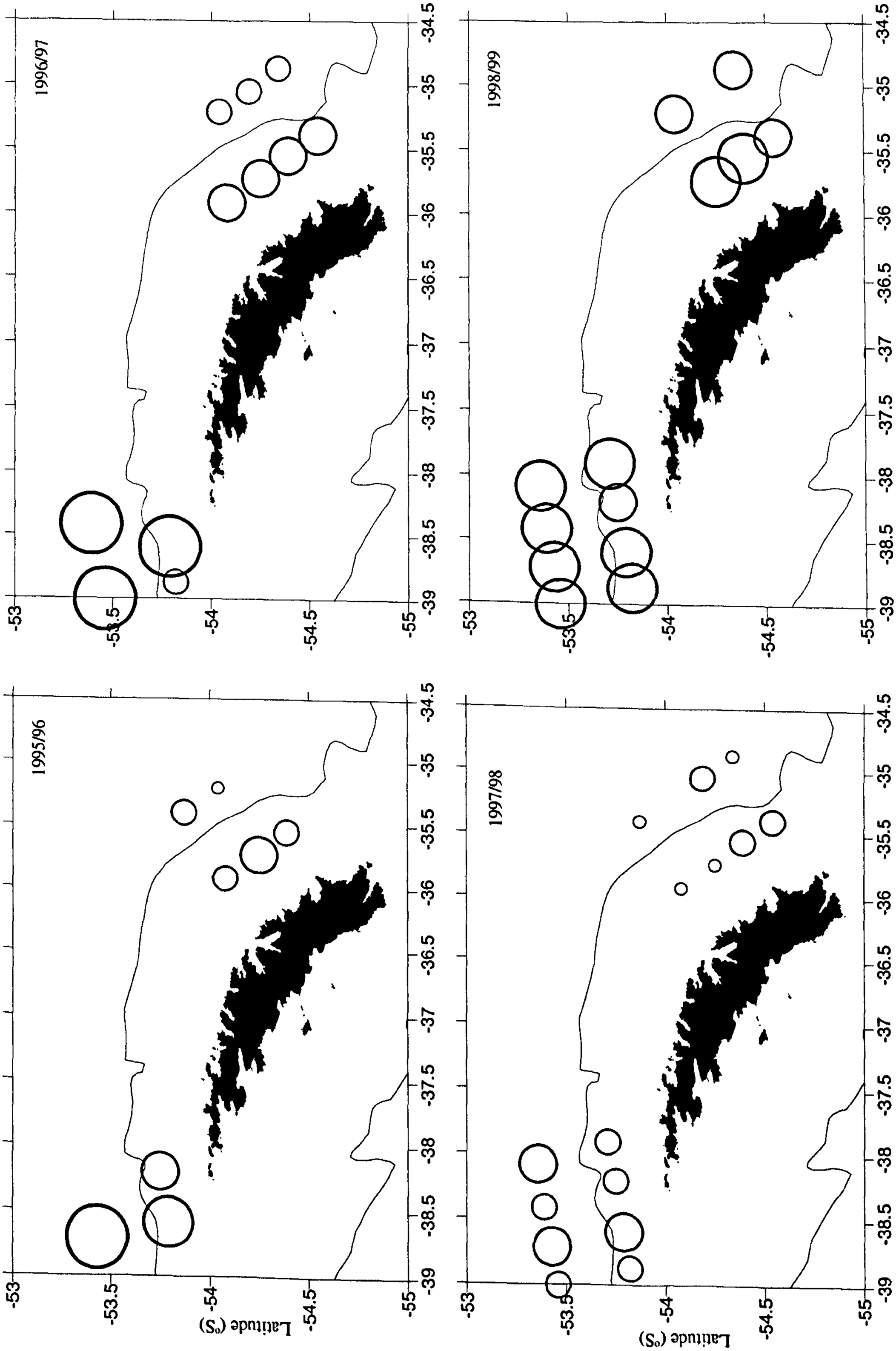
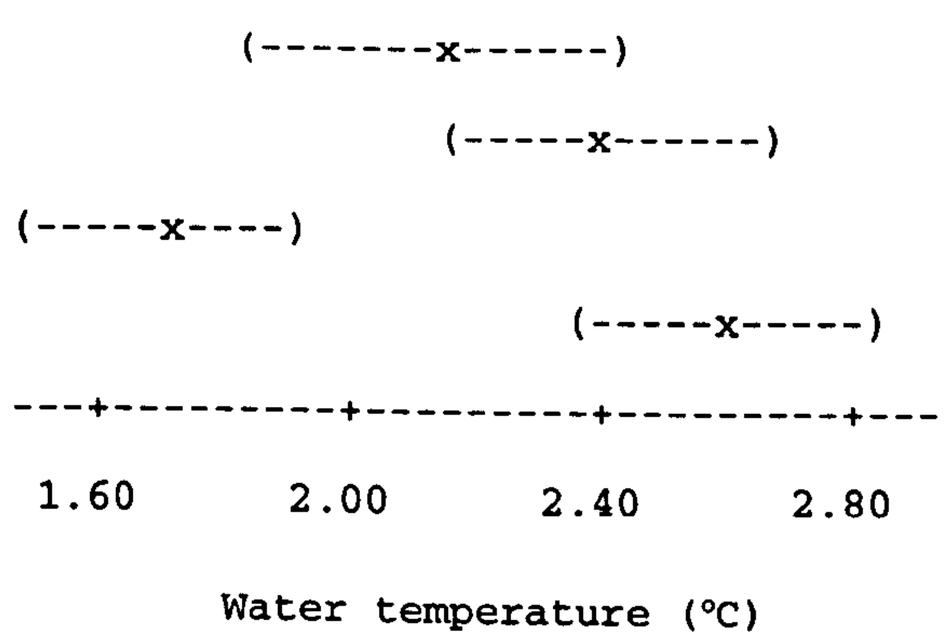

1996/97
1998/99
1995/96
1997/98
-53
-53.5
-54
-54.5
-55
-34.5
-35
-35.5
-36
-36.5
-37
-37.5
-38
-38.5
-39
Latitude (°S)
Longitude (°W)

Figure 3.5 ANOVA. Mean water temperature (0 - 60 m) ± Standard deviation (SD) in relation to a. Year F = 11.25 **, b. The eastern mesoscale box (EMB) and western mesoscale box (WMB) F = 15.98 * c. On-/Off-shelf in the EMB F = 5.35 *,* p significant at <0.05 ** p significant at <0.001. Mean (X) with individual 95% CIs(-----).

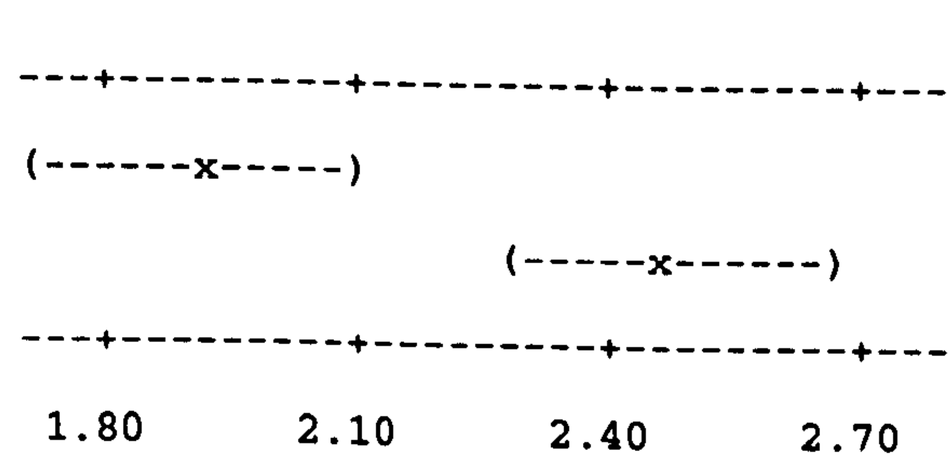
a.

Year	N	Mean	SD
1995/96	8	2.14	0.54
1996/97	11	2.40	0.63
1997/98	16	1.71	0.32
1998/99	13	2.59	0.21



b.

Area	N	Mean	SD
EMB	25	1.91	0.47
WMB	23	2.46	0.49



c.

EMB	N	Mean	SD
Off-	11	1.69	0.33
On-	14	2.09	0.50

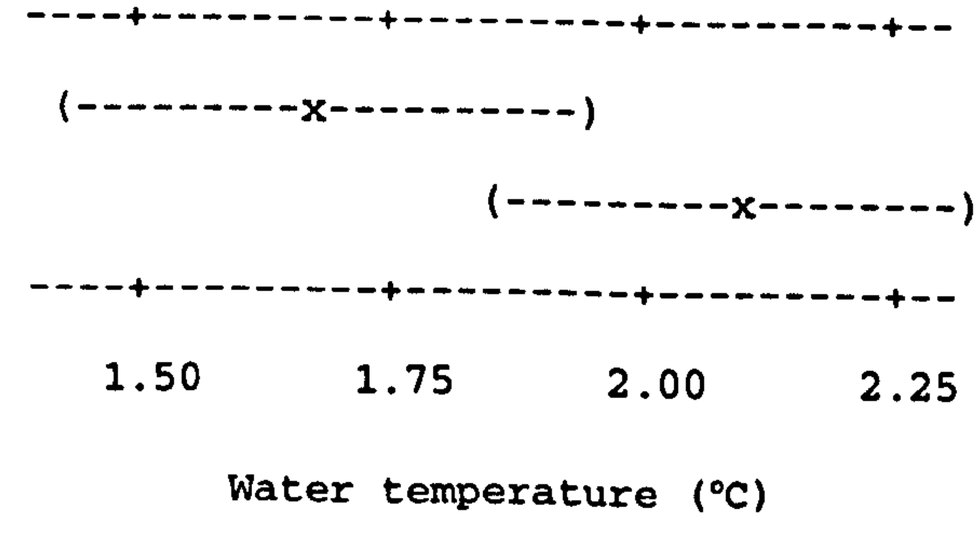


Figure 3.6 Mean silicate concentration (mol m^{-2}) integrated over the top 60 m, at each station during the four year survey.

○ 0 - 0.4, ◯ 0.4 - 0.8, ◯ 0.8 - 1.2, ◯ 1.2 - 1.6, ◯ 1.6 - 2.1 Shaded area shows the land mass of South Georgia, contour line shows the 500 m isobath.

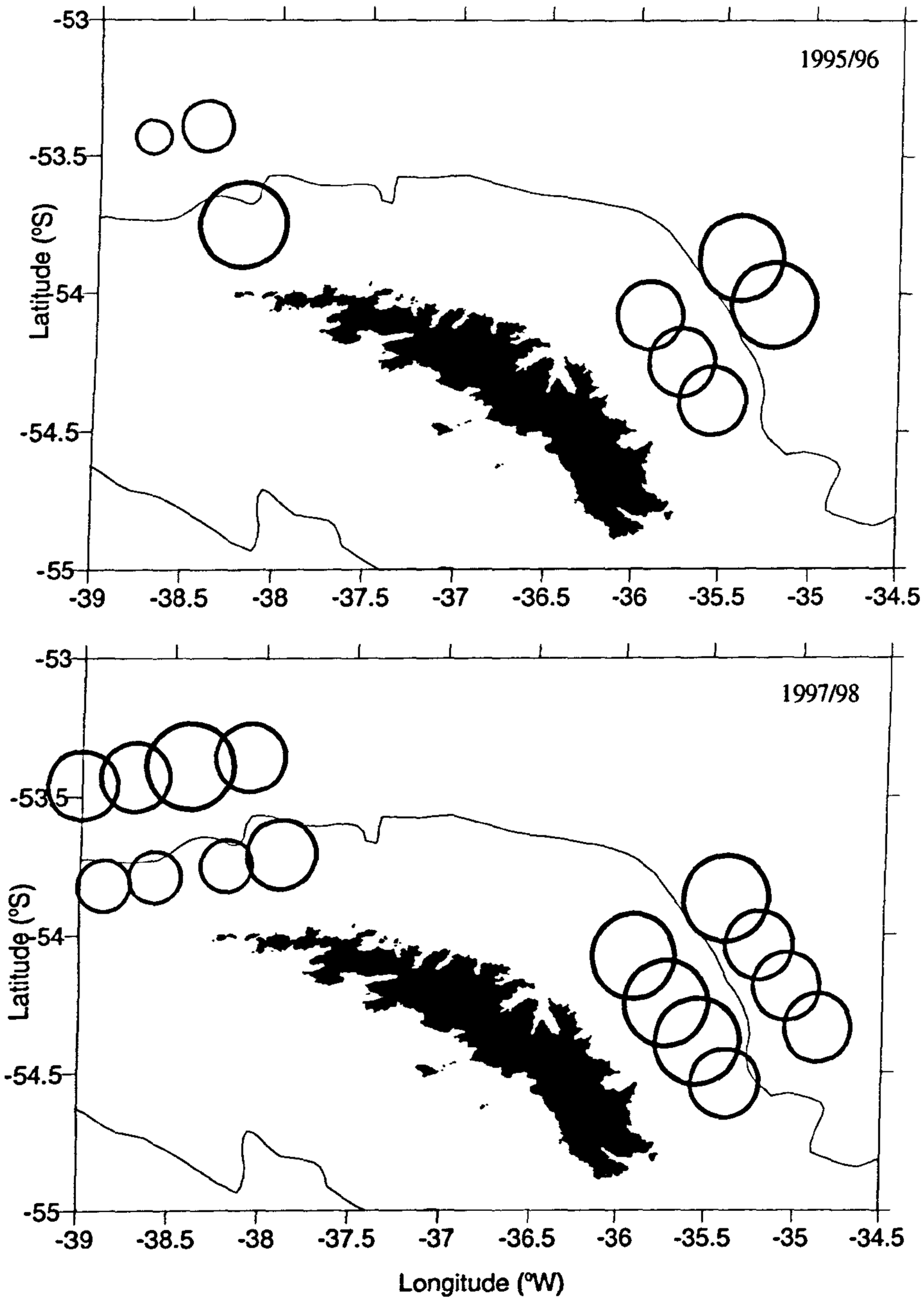

1995/96
1996/97
1997/98
1998/99
Latitude (°S)
Longitude (°W)
-53
-53.5
-54
-54.5
-55
-39
-38.5
-38
-37.5
-37
-36.5
-36
-35.5
-35
-34.5

Figure 3.6

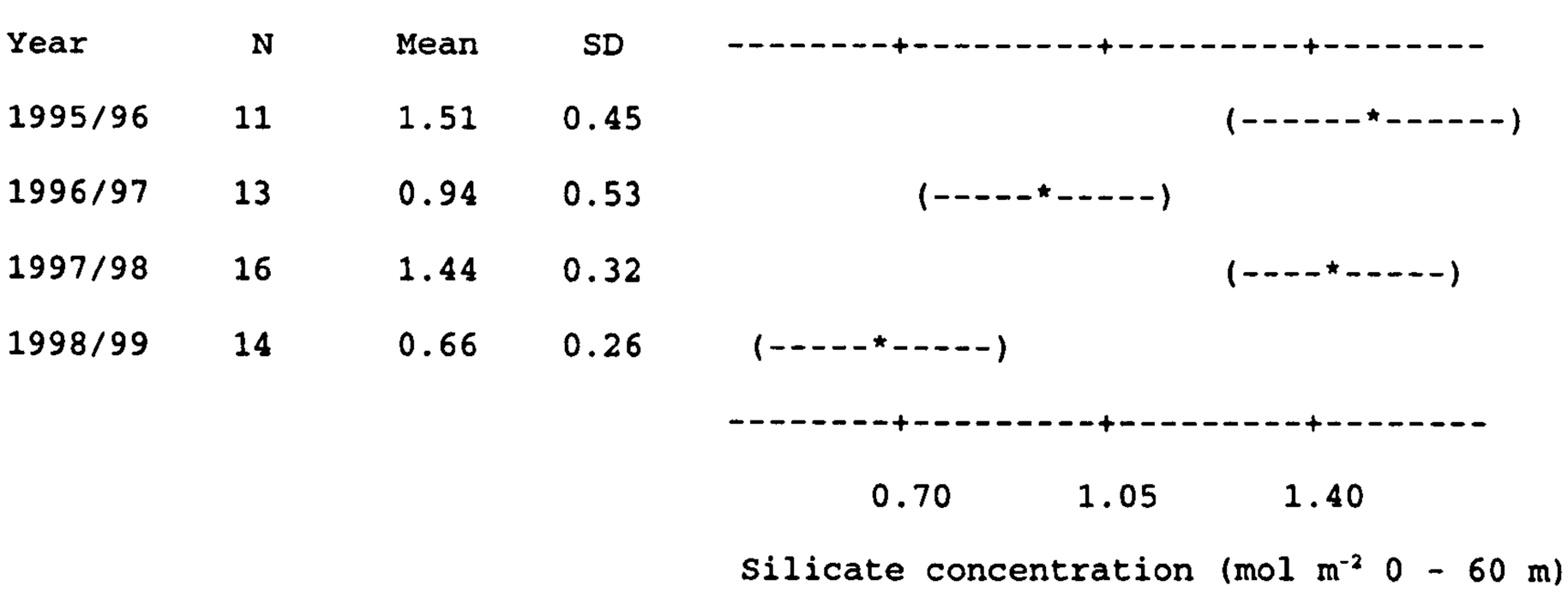
54

Lower concentrations of silicate in the top 60 m are indicative of its utilisation in the upper layers rather than a general paucity in the water column as a whole. This is evident from the vertical profiles of silicate concentration (Fig. 3.8) where higher levels, which are similar for all stations and years, are found at depth and indicate overwintering concentrations. Depletion generally occurs due to phytoplankton growth in the upper water column. The phytoplankton composition around South Georgia is generally dominated by large diatoms which use silicate in the formation of their frustules. The concentration (ng Chl *a* l^{-1}) of large diatoms (> 20 µm) in the microplankton, quantified in HPLC analysis, was regressed against silicate concentration (mol m^{-2}, 0 - 60 m) and gave a strong negative relationship (r^2 = -0.56, p <0.0001) (Fig. 3.9).

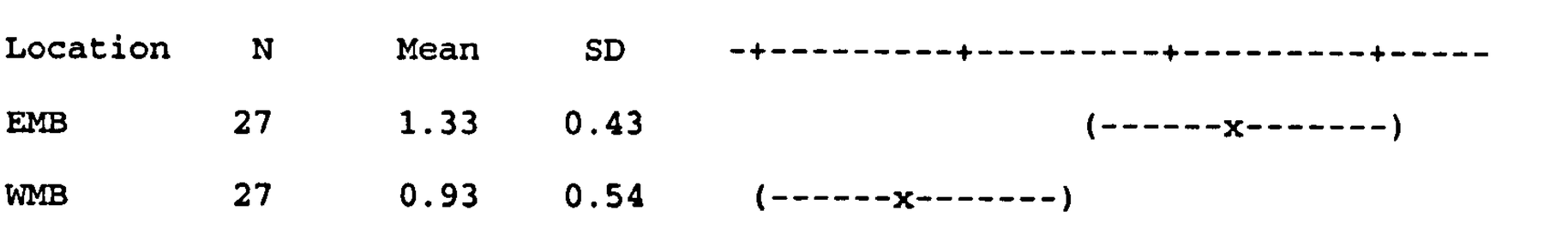
Summer nutrient dynamics may be subject to non-phytoplankton modification, such as dilution by run-off from the land mass. However typical island run-off during the survey had a negligible diluting effect on nutrient concentration (< 0.4 %, Brandon et al. 2000). The re-mineralisation of silicate in colder waters occurs over a relatively long time period compared to other nutrients, and generally does not occur within a season. Therefore the amount of silicate depletion in the upper water column was used as a proxy for phytoplankton productivity that had occurred in the recent (days to weeks) history of the water column. Silicate depletion displayed a similar pattern in relation to the island as mean water temperature. At the lower temperatures recorded, silicate concentration was around 1.8 mol m^{-2}, whereas in warmer waters silicate concentration was generally depleted to around 0.3 mol m^{-2}; simple least squares regression shows a strong negative relationship between silicate and temperature (r^2 adj = -0.52, p <0.001) (Fig. 3.10). The significance of this will be discussed in the section on the standing stock of chlorophyll *a*.

Figure 3.7 ANOVA. Silicate concentration (mol m^{-2}, 0 - 60 m) in relation to; a. Year F = 14.47** b. The western (WMB) and eastern (EMB) mesoscale boxes, F = 9.00*; c. On-/Off- shelf locations in the WMB F = 4.63*; *p significant at <0.05, ** p significant at <0.001. Mean (X) with individual 95% CIs (-----).

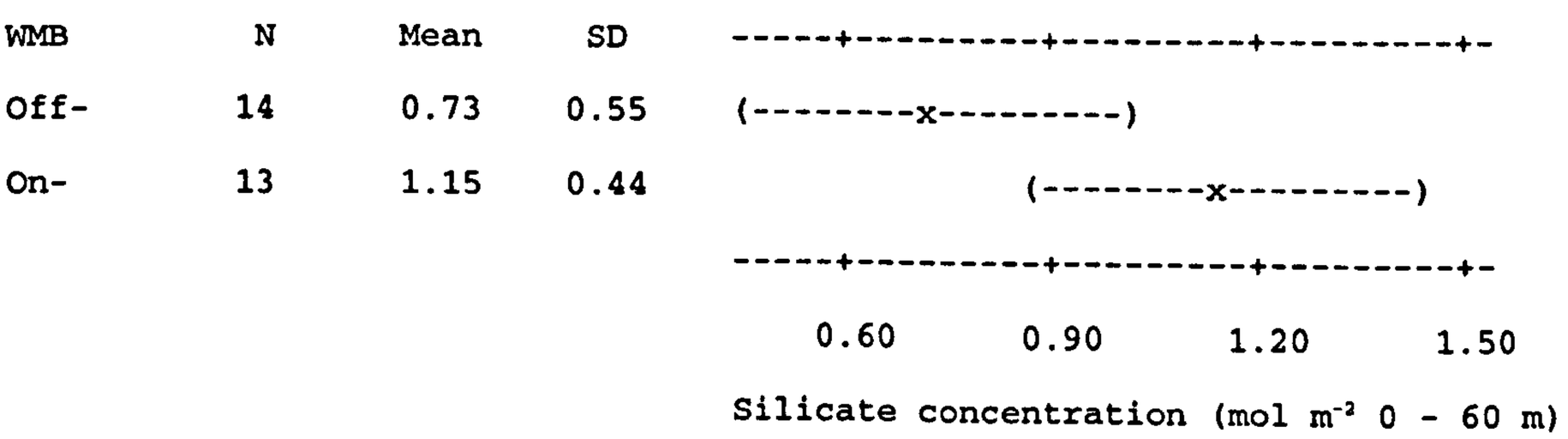
a.



b.



c.



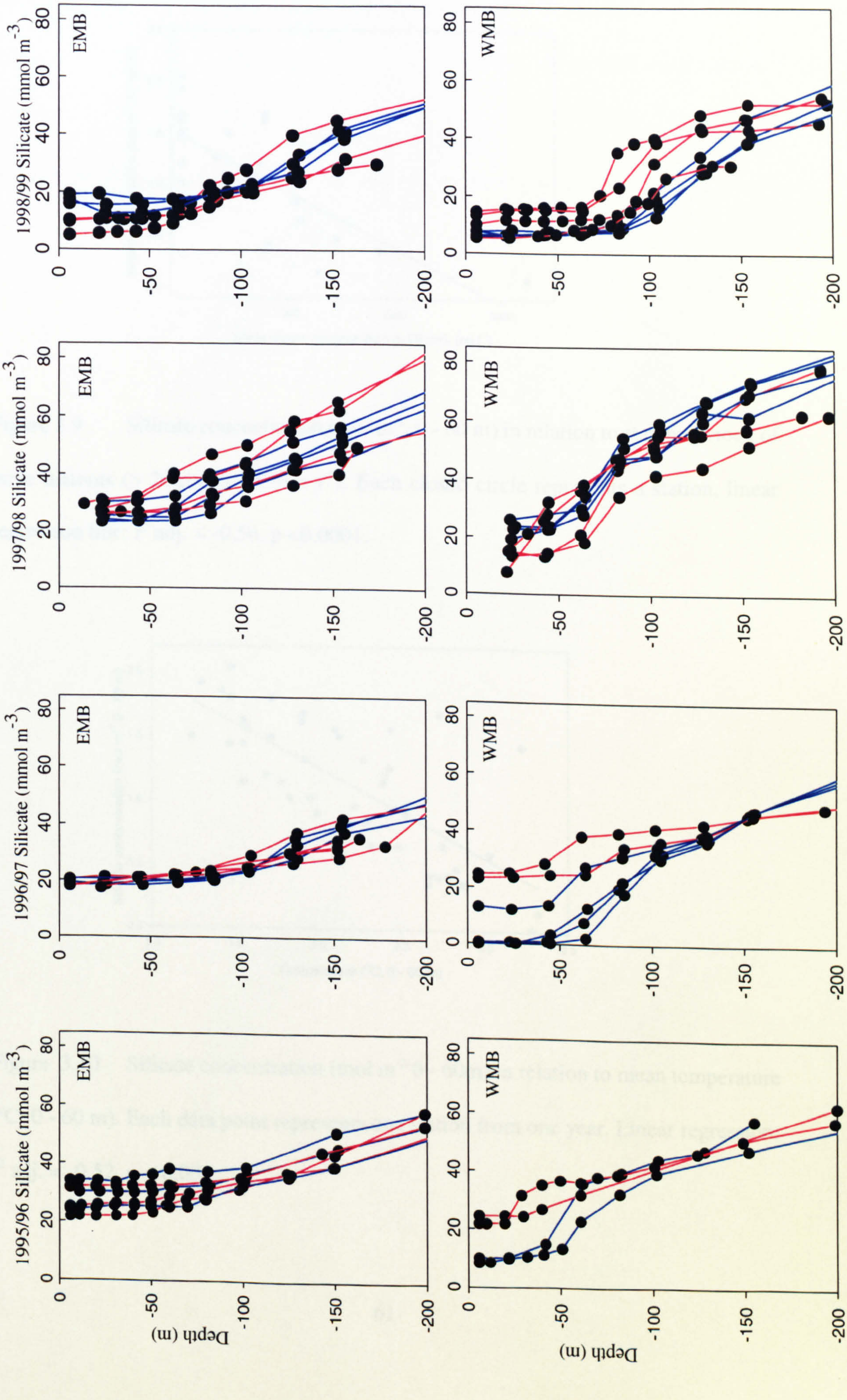
Chlorophyll a concentrations

Standing stock of chlorophyll *a* was variable between areas and years although general patterns in chlorophyll *a* concentration were evident. Mean chlorophyll *a* concentrations integrated over the top 60 m of the water column ranged from 0.24 - 10.25 g m^{-2} (Fig. 3.11). The highest concentrations of chlorophyll *a* were found during the 1998/99 season, where the mean value was 3.98 mg m^{-3} ± 2.27 (SD) (Fig. 3.12a). Higher concentrations were found in the WMB (Fig. 3.12b) and in both boxes higher concentrations were found on-shelf (Fig. 3.12c).

Chlorophyll *a* concentrations followed a similar pattern to the temperature profiles in relation to the island in so far as higher concentrations of chlorophyll *a* were generally associated with warmer water. A comparison between stations showed a weak but significant positive relationship between standing stock of chlorophyll *a* and temperature (0 - 60 m) (r^2 adj. = 0.16, p = 0.005) (Fig. 3.13a). An increase in temperature from 2 to 3°C, was associated with an approximate doubling in the standing stock of chlorophyll *a*. The three highest values for chlorophyll *a* occurred at lower temperatures than would be predicted from the regression. They originated from two on-shelf stations in the WMB where chlorophyll *a* was exceptionally high (stations 4 and 5 during 1997/98 and 4 during 1998/99). This area has been associated with the mixing of two water masses, the South Georgia Shelf Water (SGSW) and the Antarctic Zone Water (AZW), (Brandon et al. 2000). This area of up-welling may explain these anomalous data points and removing them significantly improved the fit of the regression line (r^2 adj. = 0.36, p <0.0001, Fig. 3.13b). The higher phytoplankton standing stock in this area is likely to have been present for some time (days to weeks) as the level of silicate depletion at these stations was high (r^2 adj. = -0.53, p <0.0001 Fig. 3.13c).

Figure 3.8 Silicate profiles with depth, for each station sampled during the four years. EMB Eastern Mesoscale Box, WMB Western Mesoscale Box. Circles represent spot measurements of silicate concentration (mmol m^{-3}) at each depth. Red lines indicate on-shelf profiles and blue lines the off-shelf profiles.

Figure 3.8


1998/99 Silicate (mmol m-3)
1997/98 Silicate (mmol m-3)
1996/97 Silicate (mmol m-3)
1995/96 Silicate (mmol m-3)
EMB
WMB
0
20
40
60
80
-50
-100
-150
-200
Depth (m)

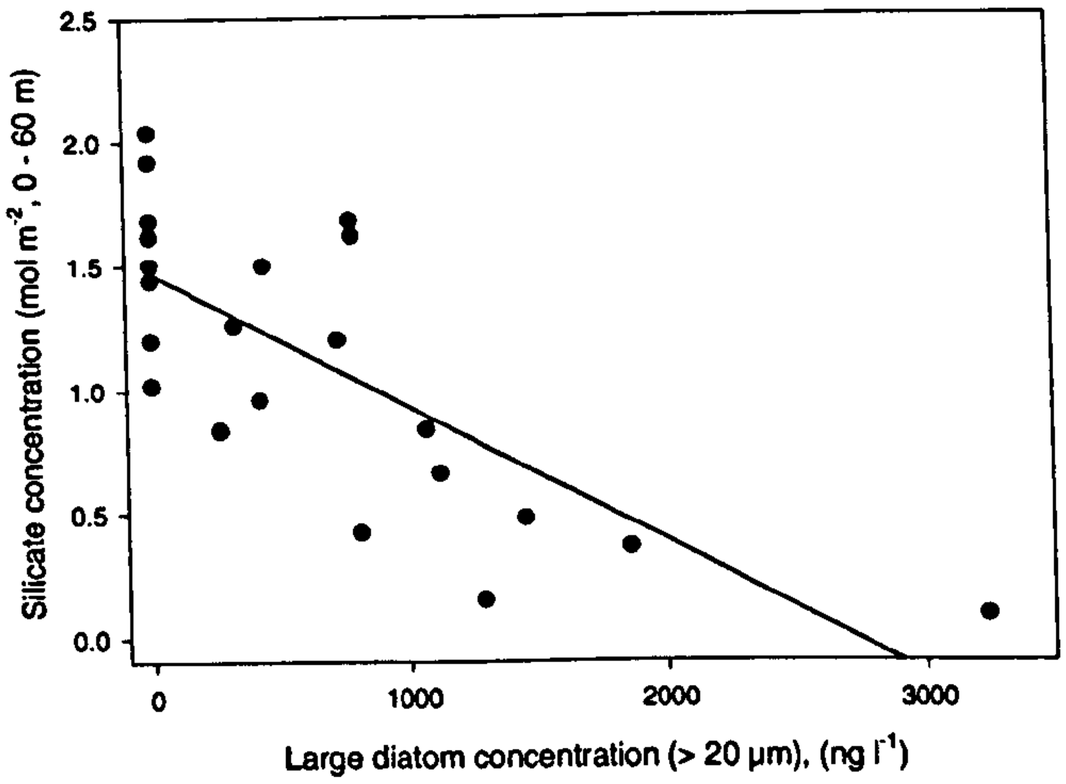


Figure 3.9 Silicate concentration (mol m^{-2}, 0 - 60 m) in relation to standing stock of large diatoms (> 20µm) (ng Chl *a* l^{-1}). Each closed circle represents a station, linear regression line: r^2 adj. = -0.56, p <0.0001.

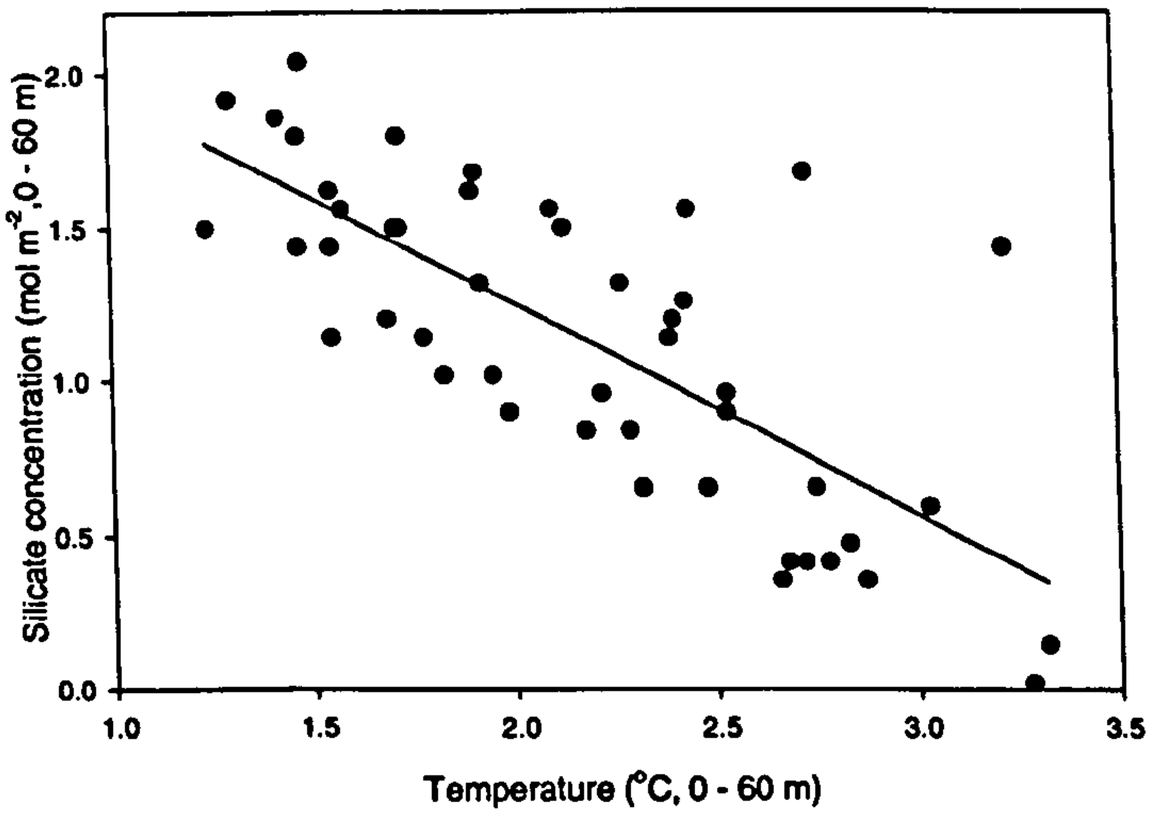


Figure 3.10 Silicate concentration (mol m^{-2} 0 - 60m) in relation to mean temperature (°C, 0 - 60 m). Each data point represents one station from one year. Linear regression: r^2 adj. = -0.52, p < 0.001.

Figure 3.11 Chlorophyll *a* concentration (g m^{-2}) integrated over the top 60 m, for all stations sampled during the survey.

Shaded area shows the land mass of South Georgia, contour line shows the 500 m isobath.

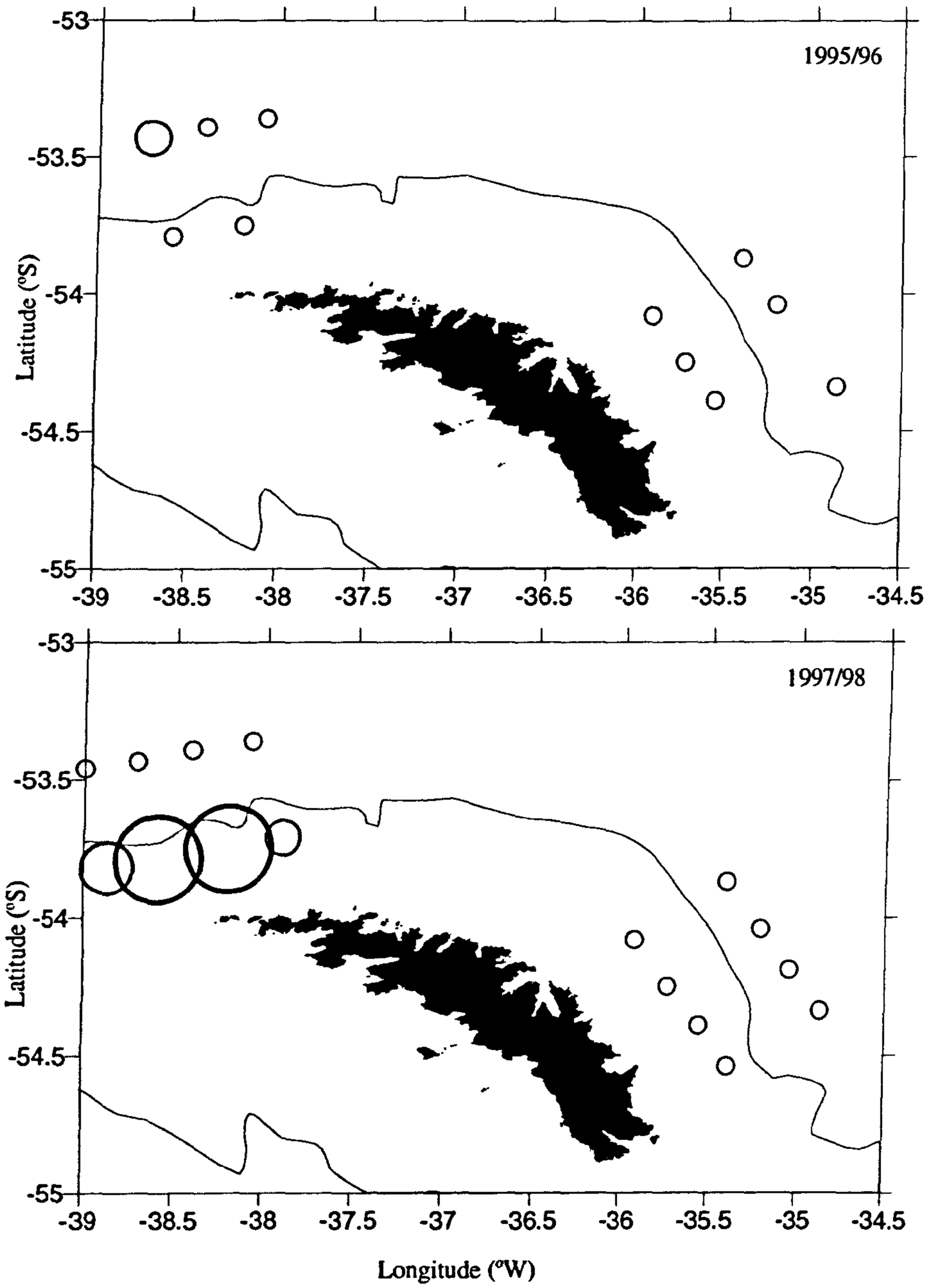
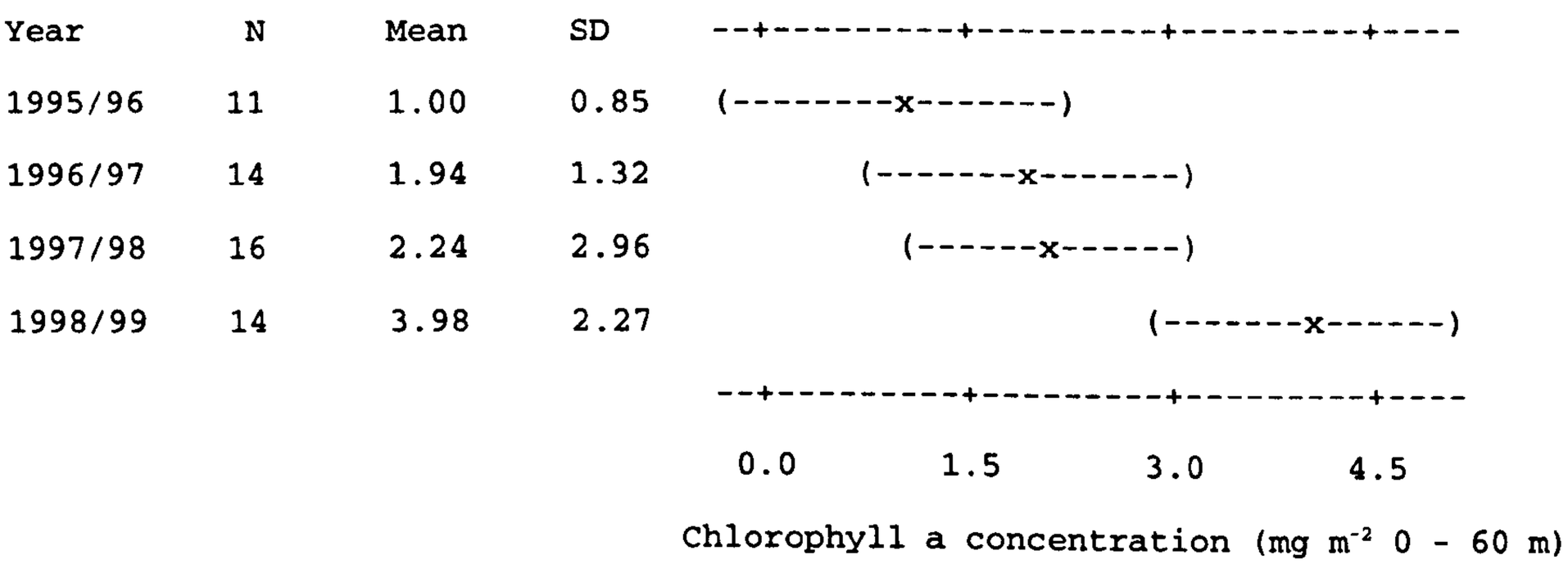

1995/96
1996/97
1997/98
1998/99
-53
-53.5
-54
-54.5
-55
-39
-38.5
-38
-37.5
-37
-36.5
-36
-35.5
-35
-34.5
Latitude (°S)
Longitude (°W)

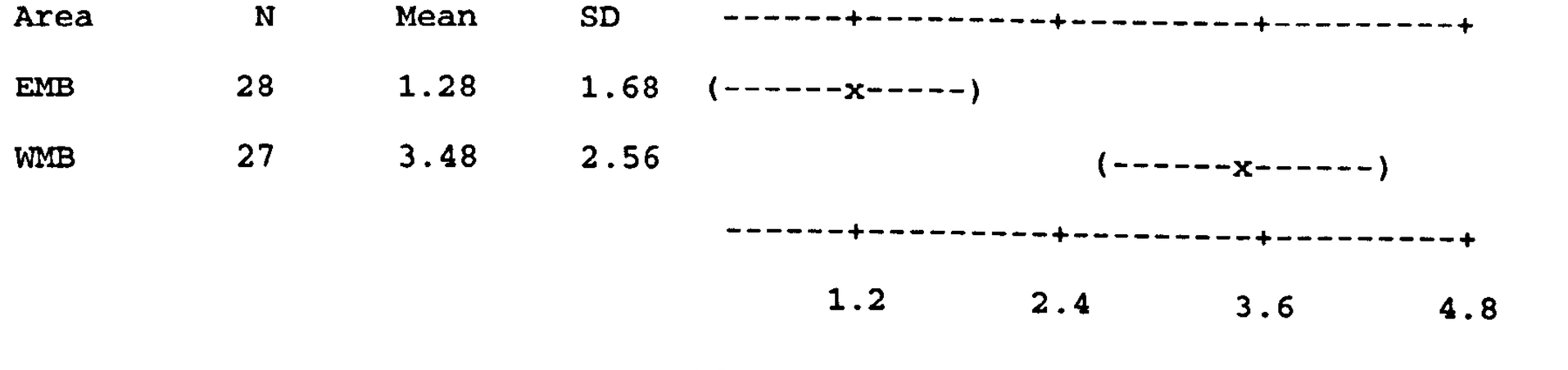
Figure 3.11

Figure 3.12 ANOVA. Chlorophyll *a* concentration (g m^{-2} 0 - 60 m) in relation to; a. Year F = 4.41*. b. Eastern and western mesoscale boxes (EMB and WMB respectively F = 15.96**; c. On-/Off- shelf F = 5.85*; * p significant at <0.05, ** P significant at <0.001. Mean (X) with individual 95% CIs (-----).

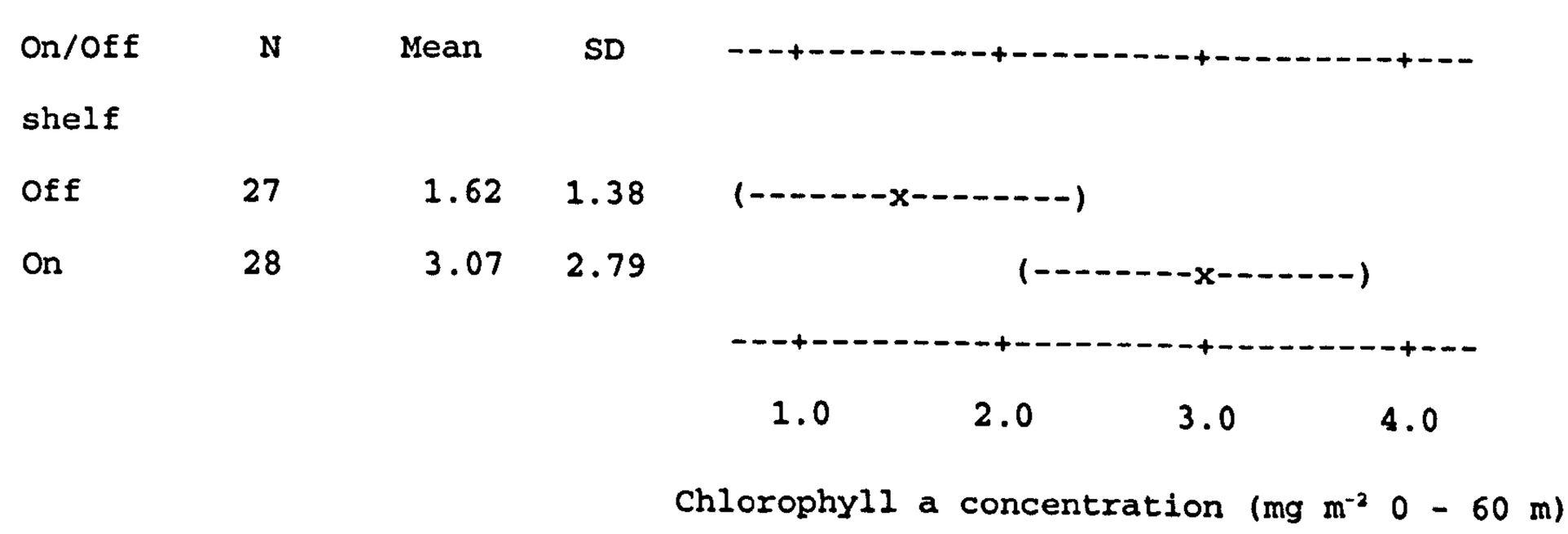
a.



b.



c.



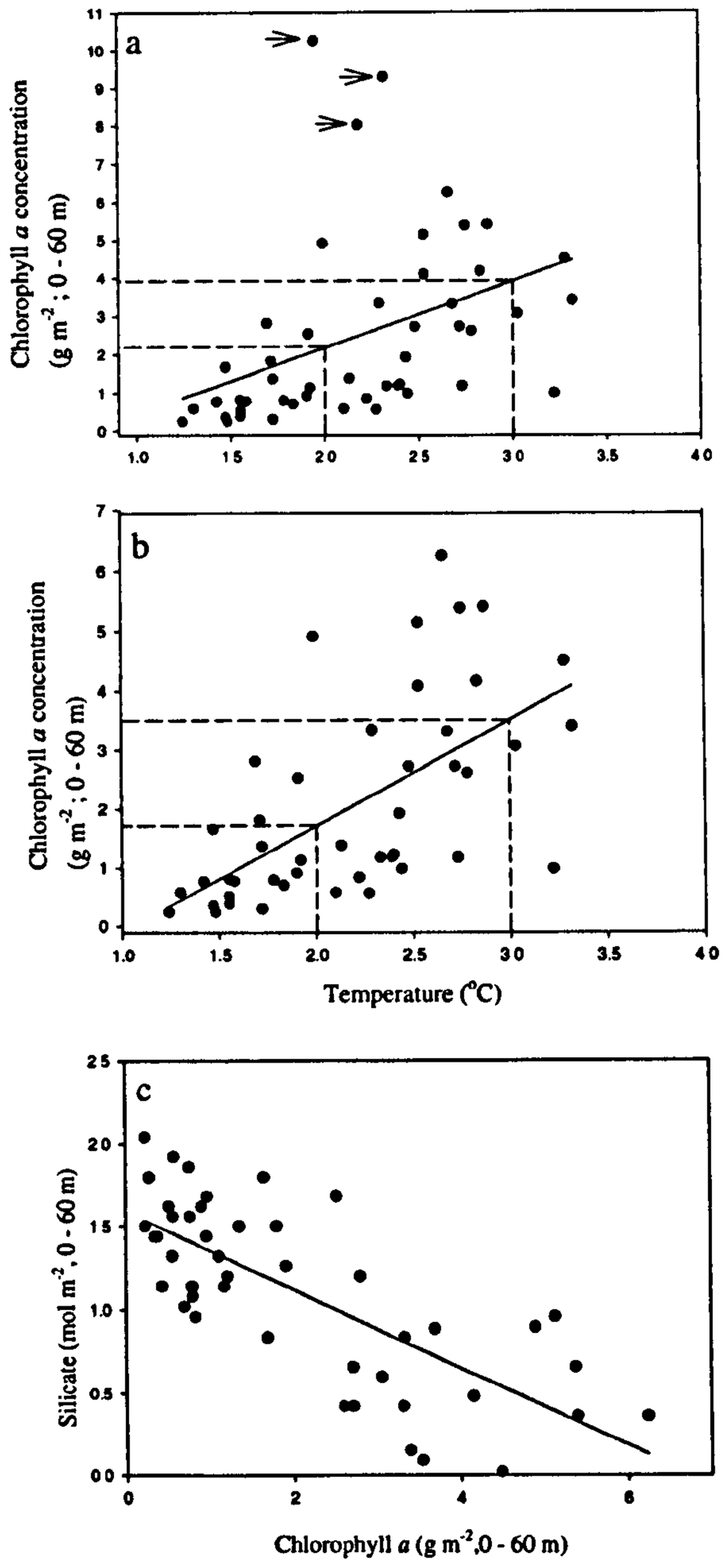


Figure 3.13 Standing stock of chlorophyll *a* (g m^{-2}, 0 - 60 m) in relation to temperature (°C). a. all stations included, least squares linear regression line r^2 adj. = 0.16, p = 0.005. Arrows indicate stations removed in b & c. b. 'Upwelling' stations removed (see text p 57 for explanation) r^2 adj. = 0.36 $p < 0.0001$. c. Chlorophyll *a* in relation to silicate (mol m^{-2} 0 - 60 m), r^2 adj. = -0.53, $p < 0.0001$.

Mixed layer depth (MLD)

The average depth of the mixed layer was far greater during 1998/99 than any of the other years (Fig. 3.14), although there were no significant differences in relation to areas. A deeper mixed layer indicates a greater amount of warming and stabilisation of the upper water column since the previous winter, and indicates the depth of circulation of nutrients and phytoplankton. The combined effect of temperature and the depth of the mixed layer on the standing stock of chlorophyll *a* was separated by regressing the mixed layer depth against temperature, and then regressing the residuals from this against chlorophyll *a* concentration. A weak positive relationship was found (Fig. 3.15), suggesting that the deeper mixed layer may enhance primary production. This is counterintuitive, as a deeper mixed layer may take phytoplankton out of the euphotic zone, hence causing light limitation (Sakshaug & Holmhansen 1986). However if vertical circulation within this zone is high, it may expose most of the phytoplankton to light levels above the compensation point for most of the time. Hence the greater pool of nutrients available for phytoplankton growth within this larger body of water may off-set any negative effects of light limitation. Whilst the Southern Ocean is generally considered to be a high nutrient low chlorophyll region, a lack of iron has been implicated as being a major limiting factor to primary production (De Barr et al. 1990, Martin et al. 1990, Buma et al. 1991). This greater nutrient pool may thus relieve some of the iron limitation normally experienced by Southern Ocean algae.

Figure 3.14 ANOVA. Mixed Layer Depth in relation to year $F = 34.43$ $p < 0.001$.

Mean (X) with individual 95% CIs (-----)

Year	N	Mean	SD	----+---------+---------+---------+--
1995/96	11	35.00	16.43	(---x---)
1996/97	13	26.54	14.91	(---x--)
1997/98	16	37.81	22.06	(--x---)
1998/99	14	86.43	12.16	(---x--)

----+---------+---------+---------+--

25 50 75 100

Mixed Layer Depth (m)

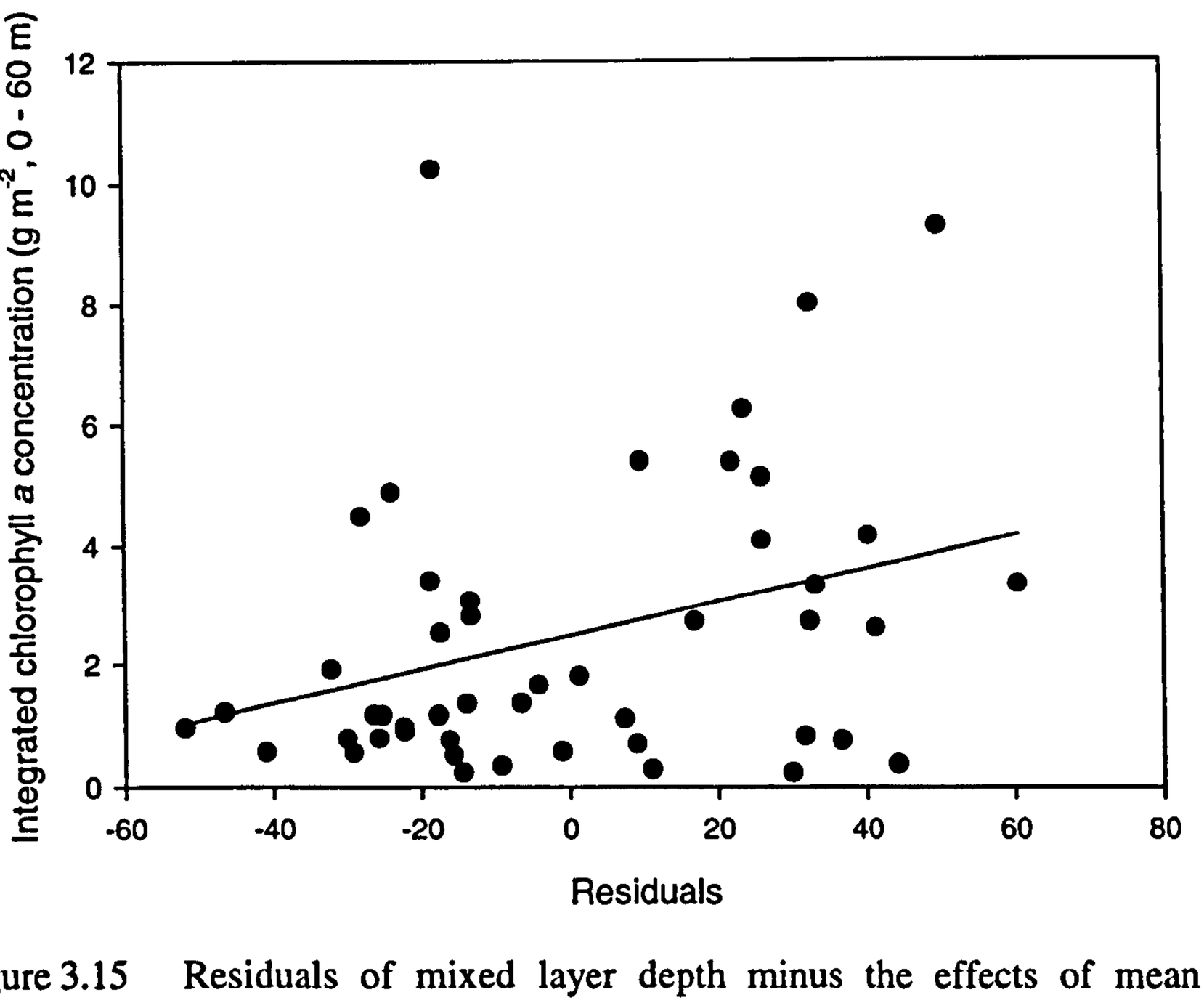


Figure 3.15 Residuals of mixed layer depth minus the effects of mean water temperature, plotted against chlorophyll *a* concentrations (see p. 67 for details) (r^2 adj. = 0.09, p = 0.019)

Krill biomass

Acoustic estimates of krill (*Euphausia superba*) biomass showed a wide range of densities from around 1 to > 2000 g m^{-2} over the stations and years studied. The data were highly skewed, and were therefore transformed ($\log_{10\,(n+1)}$) in order to normalise them. Krill concentration in the vicinity of each station for each year is shown in Fig. 3.16. The lowest krill biomass occurred during 1998/99 (Fig. 3.17a), and although very patchy, there were significant differences between the WMB and EMB (Fig. 3.17b), which was driven mainly by the lack of krill off-shelf in the WMB (Fig. 3.17c). An exceptionally high biomass of krill was found in the EMB during 1997/98 (Fig. 3.16), and was associated with the coldest water mass recorded during this survey. Simple least squares regression showed a weak negative correlation between mean water temperature (0 - 60 m) and krill biomass, (r^2 adj. = -0.14, $p < 0.005$; Fig. 3.18). At the scales used in this study, krill did not appear to have altered the food environment in any way. Regression of large and small diatoms and flagellates (ng l^{-1}) derived from the HPLC analysis showed no significant relationships with krill biomass, ($r^2 = 2.4$, $p = 0.2$; $r^2 = 0$, $p = 0.09$; $r^2 = 0$, $p = 0.9$ respectively). High densities of krill were never found associated with high concentrations of chlorophyll *a*.

Copepod abundance

Total copepod abundance (Fig. 3.19) also showed variability between areas and years. Abundance ranged from 14,000 to 337,000 individuals m^{-2} (0 - 200 m) and one of the greatest difference between years in the total abundance of all copepod species was found during 1998/99, where they were generally more numerous at all stations sampled (Fig. 3.20a). Abundance was generally higher in the WMB compared to the EMB (Fig. 3.20b), with a higher abundance found off-shelf in the WMB (Fig. 3.20c).

Figure 3.16 Krill biomass ($\log_{10 (n+1)}$) g m^{-2} integrated over the top 250 m.

○ 0 - 1.5, ◯ 1.5 - 3, ◯ 3 - 4.5, ◯ 4.5 - 6 , ◯ 6 - 8. Shaded area shows the land mass of South Georgia, contour line shows the 500 m isobath.

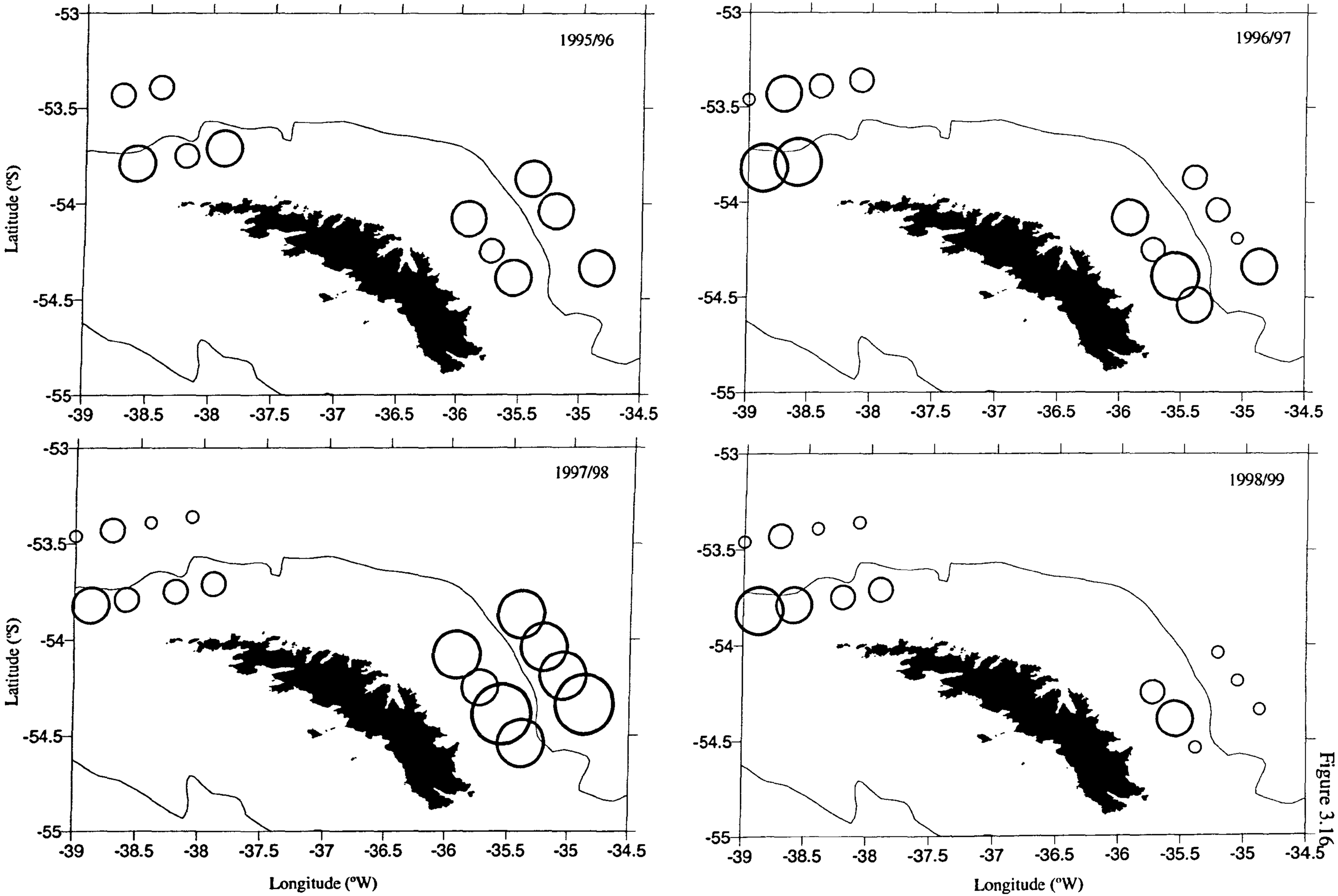
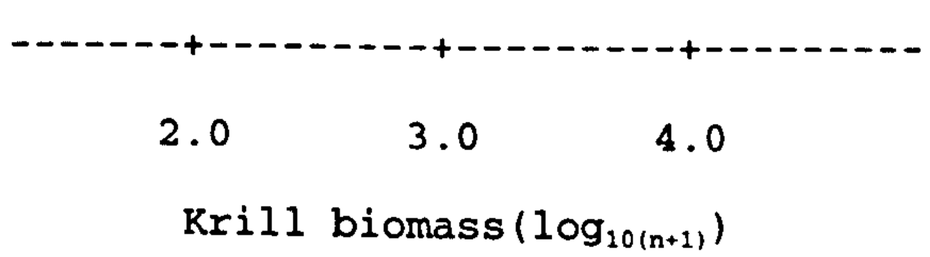

1995/96
1996/97
1997/98
1998/99
-53
-53.5
-54
-54.5
-55
-39
-38.5
-38
-37.5
-37
-36.5
-36
-35.5
-35
-34.5
Latitude (°S)
Longitude (°W)

Figure 3.16

Figure 3.17 ANOVA. Krill ($log_{10(n+1)}$) in relation to; a. Year F = 2.86* b. Eastern and western mesoscale boxes (EMB and WMB respectively) F = 5.67*, c. Area F = 4.95*. * P significant at <0.05, ** P significant at <0.001. Mean (X) with individual 95% CIs (-----)

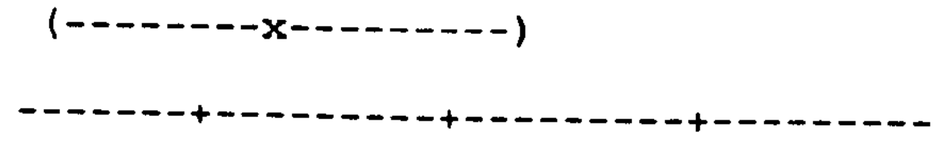
a.

Year	N	Mean	SD
1995/96	11	3.22	0.55
1996/97	14	3.20	1.35
1997/98	16	3.72	2.10
1998/99	14	2.17	1.19



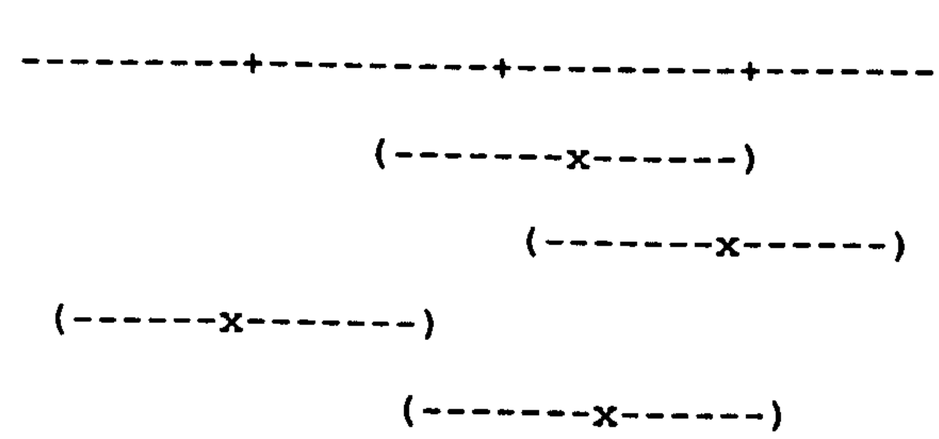
b.

Area	N	Mean	SD
EMB	28	3.56	1.67
WMB	27	2.606	1.27



c.

Location	N	Mean	SD
EMB off-	14	3.26	1.90
EMB on-	14	3.86	1.41
WMB off-	14	1.91	0.87
EMB on-	13	3.36	1.22



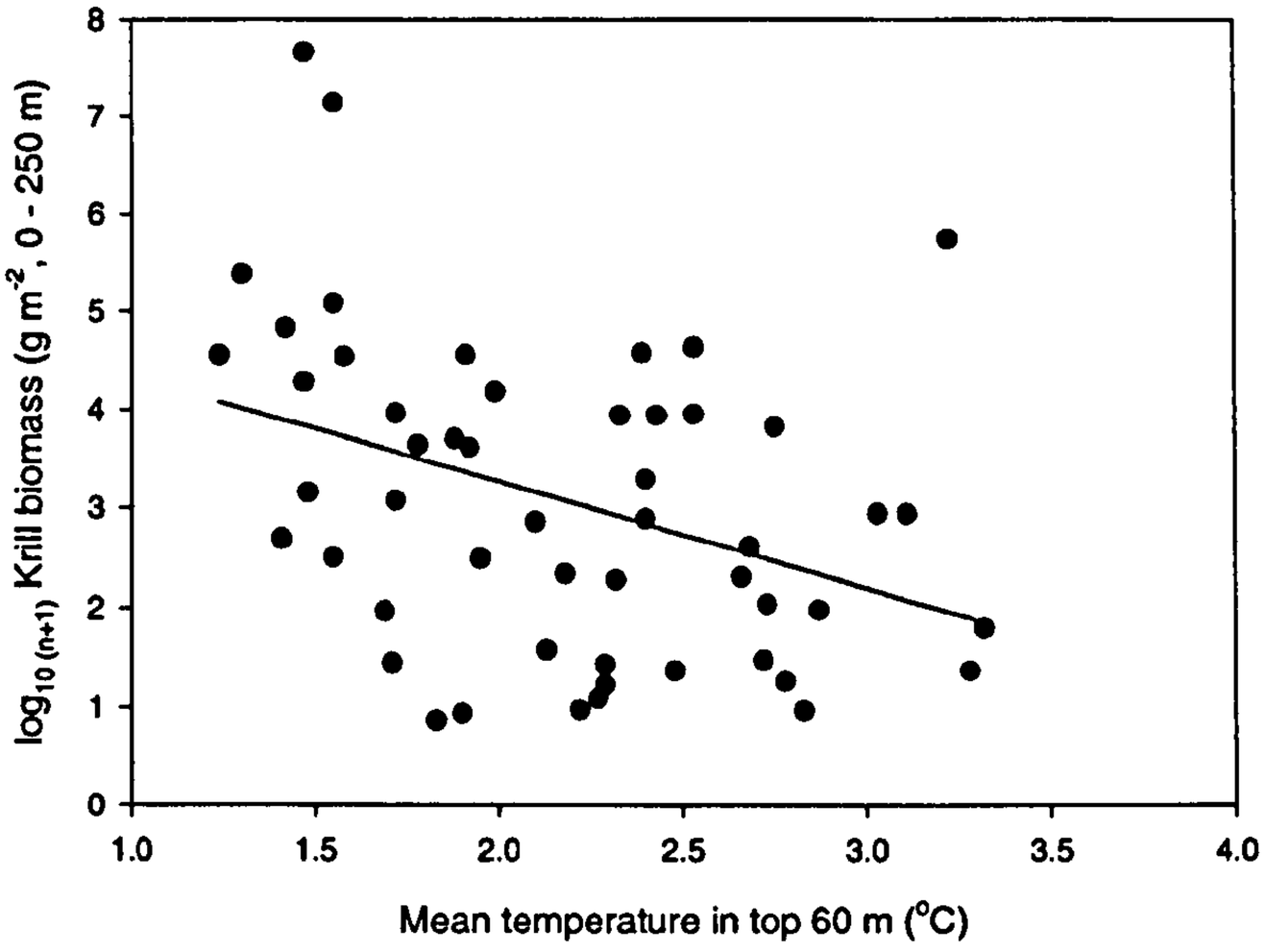


Figure 3.18 Krill biomass in relation to mean temperature in the top 60 m. Each circle represents one station during one of the years. Regression line (r^2 adj. -0.12, p = 0.006).

Figure 3.19 Total copepod abundance (ind m^{-2}, 0 - 200 m) in relation to station and year.

○ 14000 - 20000, ◯ 20001 - 50000, ◯ 50001 - 100000 , ◯ 100001 - 200000, ◯ 200001 - 440000. Shaded area shows the land mass of South Georgia, contour line shows the 500 m isobath.

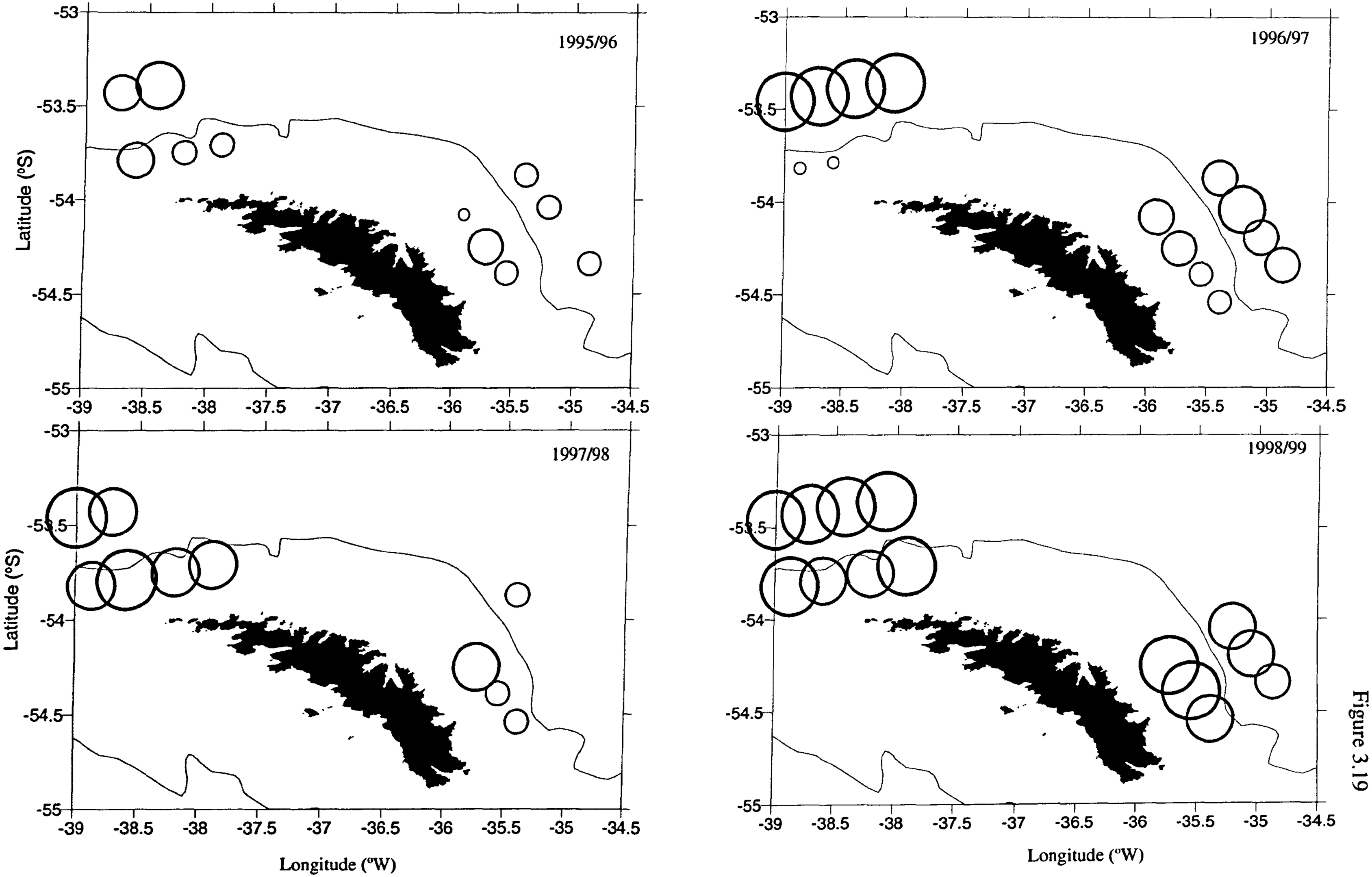

1995/96
1996/97
1997/98
1998/99
-53
-53.5
-54
-54.5
-55
-39
-38.5
-38
-37.5
-37
-36.5
-36
-35.5
-35
-34.5
Latitude (°S)
Longitude (°W)

Figure 3.19

Figure 3.20 ANOVA Total copepod abundance in relation to; a. Year F = 9.07 P = 0.000, b. Eastern and western mesoscale boxes (EMB/WMB respectively) F = 20.21 P = 0.000, c. Location F = 8.94 P = 0.000. Mean (*) with individual 95% CI (-----)

a.

Year	N	Mean	SD	---------+---------+---------+------
1995/96	11	52255	32302	(------*-----)
1996/97	14	117664	106308	(-----*----)
1997/98	16	118295	71229	(----*----)
1998/99	14	219111	90979	(----*-----)

---------+---------+---------+------

80000 160000 240000

Copepod abundance (Ind m^{-2} 0 -200 m)

b.

Area	N	Mean	SD	-+---------+---------+---------+-----
EMB	28	80280	56318	(-----*-----)
WMB	27	182761	106161	(------*-----)

-+---------+---------+---------+-----

50000 100000 150000 200000

Copepod abundance (Ind m^{-2} 0 -200 m)

c.

Location	N	Mean	SD	------+---------+---------+---------+
EMB off-	14	73841	30771	(------*-----)
EMB on-	14	86719	74507	(-----*------)
WMB off-	14	217331	98538	(-----*-----)
WMB on-	13	145531	104900	(------*-----)

------+---------+---------+---------+

70000 140000 210000 280000

Copepod abundance (Ind m^{-2} 0 -200 m)

3.4 Summary

There was considerable variation in all physical, chemical, and biological parameters measured during the present study, which is a characteristic of the South Georgia ecosystem and has been described previously. Such heterogeneity in the hydrography and nutrients has been documented by Deacon (1933) and Whitehouse et al. (1996). Similar variation in the phytoplankton has been documented by Hart (1934) and Priddle et al. (1986) and for zooplankton by Hardy & Gunther (1935) and Atkinson (1989). The present data show that the western end of the island was generally warmer and supported a higher phytoplankton biomass than the eastern end. This was evident both in the levels of chlorophyll *a* found in the west, and the extent of silicate depletion there. Copepod abundance was also generally elevated in this area.

The data suggest that during the period of this study the water mass surrounding the island always lies to the north of the SACCF. The silicate profiles showed similar concentrations at depth at all stations, which suggests that silicate replete Weddell Sea water did not significantly affect the physical environment during this survey. The θ max at the 500 m depth horizon did not drop below 1.8°C, again suggesting that colder water from the southern edge of the ACC did not affect the survey area at the time of sampling. Temperature differences between years thus appeared to be driven by seasonality, with annual large-scale variability caused by factors other than water mass change. Nutrient variation (silicate depletion) on the other hand appeared to be due to small scale processes associated with phytoplankton production.

The higher silicate concentrations associated with colder water suggests that phytoplankton growth around South Georgia is either limited by low temperature or some allied factor. Reay et al. (1999, 2001) report a tripling in phytoplankton growth rate

when temperature was raised between 3 and 6°C above the ambient sea water temperature. They ascribed these large differences in production to the reduced ability of micro-algae to utilise nutrients at the lower temperatures. Southern Ocean phytoplankton are generally psychrotolerant, with the temperature optima for growth being in excess of those they usually encounter (Tilzer et al. 1986, Smith and Harrison 1991). There was no evidence, at the scales worked on in this study, to suggest that krill grazing was affecting the concentration or composition of the phytoplankton, and high densities of krill were never found associated with high concentrations of chlorophyll *a*.

In subsequent chapters the environmental factors described here will be considered in relation to the growth and development of copepods. Although the parameters have been described separately here, the fact that some may act synergistically must also be take into account. In this respect the close relationship between temperature and silicate may confuse which factor is the most important in determining differences in growth and development. In these cases relationships with either temperature or silicate are described selecting the predictor variable that has a sound physiological basis.

Chapter 4 Moulting rates and development times of *Rhincalanus gigas* and *Calanoides acutus*

Data from Chapters 4 - 8 also presented in:

Shreeve RS, Ward P (1998) Moulting and growth of the early stages of two species of Antarctic copepod in relation to differences in food supply. Mar Ecol Prog Ser 175:109-119

Shreeve RS, Ward P, Whitehouse MJ (2002) Copepod growth and development around South Georgia: relationships with temperature, food and krill. Mar Ecol Prog Ser 233: 169 - 183

4.1 Introduction

Copepod growth is accompanied by a periodic moulting of their exoskeleton. Copepods have a well defined sequence of growth stages; the development of the egg, six naupliar and six copepodite stages. Moulting from one stage to another, which is under hormonal control, essentially consists of four main phases: premoult, moulting, postmoult and intermoult. During premoult the exoskeleton is digested away by the epidermis, and a new epicuticle and exoculticle are secreted. The individuals are then ready to undergo the relatively rapid process of moulting. The body swells with the uptake of water, which may represent up to half of the premoult body mass, and the old exoskeleton is shed. During postmoult the endocuticle is secreted and hardening takes place around the water-swollen body. As the new exoskeleton hardens, excess body water is eliminated, and the soft tissues shrink away from the now slightly over-sized

exoskeleton. This allows for somatic growth during the intermoult period until the exoskeleton once again restricts further growth. The whole process is then repeated until the copepod reaches maturity, at which point moulting ceases and growth is channelled predominantly into reproduction. The physiological process of moulting actually occurs for over 70 % of the time between successive moults, and in this sense moulting can be viewed as a process that affects copepods for much of their juvenile life rather than a process that interrupts the 'normal' existence of the copepod.

In this study the period between the moulting into successive stages is of interest, because it gives an estimate of the stage duration. The combined stage duration for each stage in the life cycle of a copepod species is thus equivalent to the generation time. The time spent in each development stage relative to embryonic development has been shown to vary between species, but essentially three conceptual models have been proposed to describe the development of copepods. They are referred to as equiproportional, isochronal and sigmoidal development and are described below. The typical form of each development is shown in Fig. 4.1.

Equiproportional development was first described by Corkett (1984) for development whereby each stage would occupy the same proportion of time relative to egg development at any given temperature. Thus, given that the egg development times are determined at three or more temperatures, a Bělehrádek's function relating embryonic duration to temperature can be fitted to the data. Use of this function in conjunction with experimentally determined development times of older stages at a single temperature can predict development times of older stages at any selected temperature.

Isochronal development was described by Miller et al. (1977) whereby all stages

have virtually the same duration, as seen in *Acartia* sp. Therefore it is only necessary to estimate the duration of one stage at a particular temperature to describe the generation time for that species.

Sigmoidal development was described by Peterson (1986) for development when the early nauplii and copepodite stages have a markedly shorter duration than the later copepodite stages, a development pattern which is seen in *Calanus marshallae.*

A fourth category has been proposed, namely 'non-conformist' in which the development does not conform to those described above (Mauchline 1998).

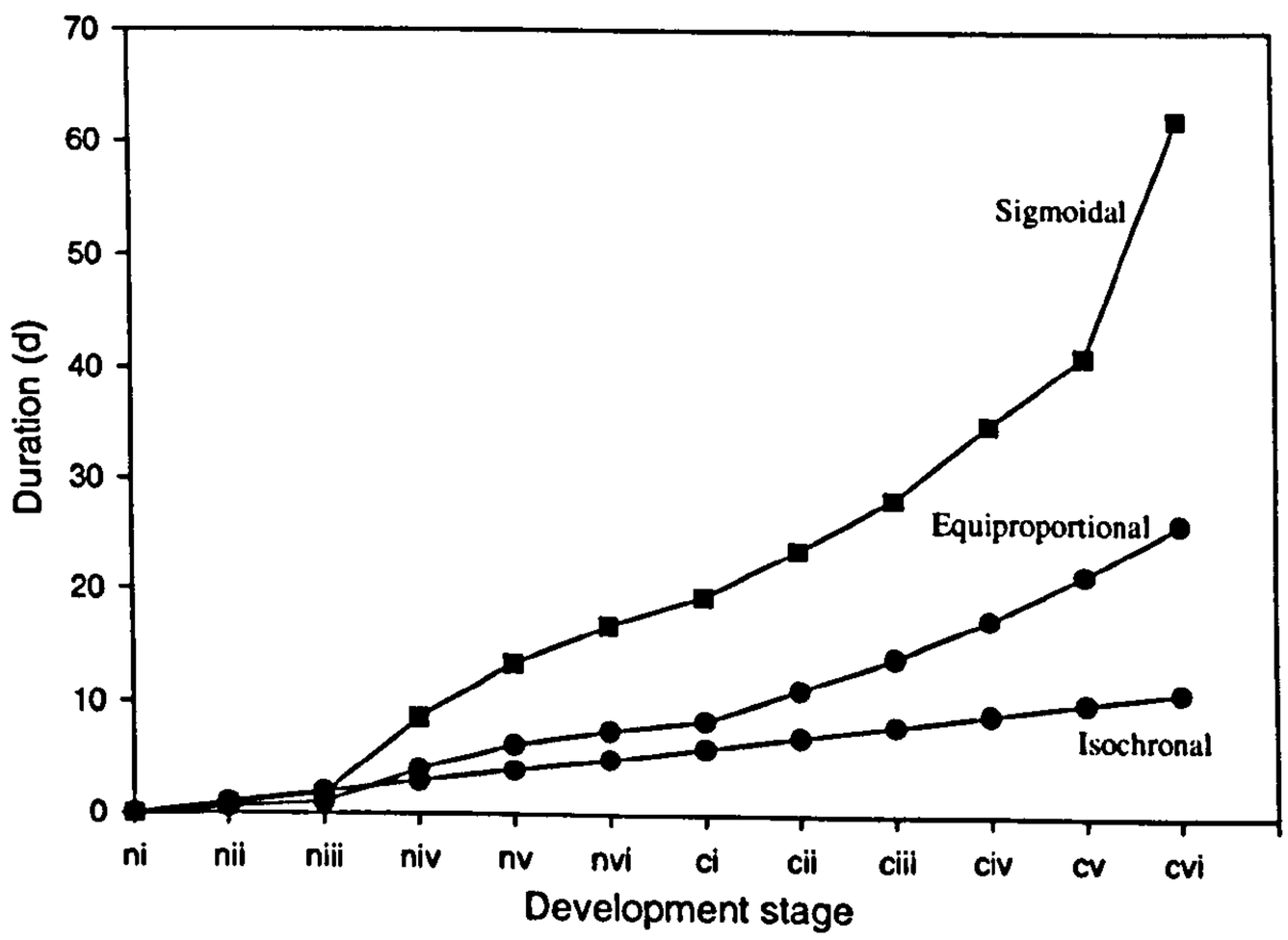


Figure 4.1 Typical forms of Isochronal, Equiproportional and Sigmoidal development. Development stage against cumulative duration, data from Miller et al. (1977), Thompson (1982) and Peterson (1986) for each mode of development respectively. Naupliar 1 - 6 (ni - nvi) and copepodite stages I - VI (ci - cvi).

The effects of temperature and food on the development of copepods have received much study attention. From work carried out on *Calanus pacificus* and *Pseudocalanus sp.* Vidal (1980b) suggested that, at lower temperatures, stage durations were increased and the development of younger stages was retarded proportionally more than that of the older stages. He also found that development was retarded at very low food concentrations, and suggested that older stages and larger species would be affected more, because the critical food concentration for growth and development increased with increasing body size. The effect of lower temperatures increasing the generation time of copepods has also been demonstrated for *Acartia clausi* (Landry 1975), *Pseudocalanus elongatus* and *Calanus* sp. (Thompson 1982) and *Calanus marshallae* (Peterson 1986).

These combined effects of temperature and food concentration on development rate have often obscured which of the two is the most important component. In this respect embryonic duration has been used to study the effects of temperature alone, since the development rate of eggs is not limited by the immediate presence or absence of food. Such studies have shown that lower temperatures do significantly retard development in *Calanus sp.* (Thompson 1982) and *Pseudocalanus sp.* (McLaren 1965). Indeed, the generation time of copepods at higher latitudes is profoundly protracted in comparison to those found in temperate waters. *Calanus finmarchicus*, one of the most studied temperate species of copepod, may complete its life cycle in as little as 30 days, whereas similar sized copepods of higher latitudes, such as *Calanus simillimus* from the Southern Ocean and a northern counterpart *Calanus glacialis* may take a year to complete their life cycles.

The life cycles of the two key species used in this study, *Rhincalanus gigas* and *Calanoides acutus*, described by Ward et al. (1997), and Atkinson et al. (1997), generally suggest a one year life cycle in the northern extent of their ranges, which may extend over two years in the populations found at higher latitudes. In this study the development times of a range of copepodite stages of *Rhincalanus gigas* and *Calanoides acutus* are presented. These help to refine the conceptual models of the life cycles of these two species.

4.2 Material and Methods

Sample collection

Plankton samples were collected from each station as described in Chapter 3. Once onboard the samples were immediately diluted in approximately 20 l of ambient sea-surface water in an opaque bucket. The sorting of live animals for experimental work then took place and was conducted within three hours post capture. During this time the temperature was maintained as close as possible (within 1°C) to the ambient sea surface temperature.

Moulting rate experiments.

Individual copepodite stages of *Rhincalanus gigas* and *Calanoides acutus* were removed under the dissection microscope using a wide bore pipette. Batches of 30 individuals of the same species and stage were placed in 2.5 l jars of 0.2 µm filtered sea water and were incubated for 48 h under constant low illumination at ambient sea surface temperature. Water used to incubate the copepods during the moulting rate experiments

was collected from a pumped non-toxic sea water supply from an inlet at 7 m depth in the ships hull. Filtration took place by pumping water through a series of Balston ® filters of pore size 25, 1 and 0.2 μm, to remove phytoplankton, predators and bacteria. The rationale behind this was to maintain the experimental conditions as constant as possible between stations. Previous work suggested that within a 48 hour period post capture, copepods would continue to develop at the same rate even in the absence of food (Miller et al. 1984). One potential problem with filtration is a possible reduction of oxygen concentration in the water; this may stress the copepods and change their physiology, potentially affecting their moulting rates. With this in mind the concentration of dissolved oxygen in the sea water before and after filtration was measured with a couloximeter, (details in Appendix 1). Sea water post-filtration was found to be 97 % saturated - an increase of 2 %. During filtration the temperature of the seawater rose by 0.5°C, so prior to use in the experiments the filtered sea water was chilled back down to ambient temperature. At the end of the 48 hour incubation period the samples were filtered down and individual stages counted. Moulting rates were calculated according to equation 3 described in Chapter 2:

$$MR_i = \frac{N_{f,(i+1)}}{N_{s,i}}$$

where MR_i is the moulting rate of stage i, $N_{s,i}$ is the number of animals at stage i at the start of the incubation, and $N_{f,(i+1)}$ is the number of animals which had moulted to stage i+1 by the end of the incubation. To calculate the moulting rate d^{-1}, MR_i was then divided by the length of the incubation in d. Stage durations (d) were calculated as the reciprocal of the moulting rates.

4.3 Results

Calanoides acutus stage CI was never found in sufficient numbers in the plankton samples taken around South Georgia to facilitate their incubation in moulting rate studies. However a study in the Scotia Sea during austral summer 2000 provided an opportunity to estimate their stage duration. For details see Appendix II. Most copepodite stages of *Rhincalanus gigas* and *Calanoides acutus* were however present in sufficient numbers to conduct moulting rate experiments. At no point however, did moulting occur in stage CV of either species during this summer survey, despite incubating over 4000 individuals of *Calanoides acutus* and over 400 of *Rhincalanus gigas*.

Individuals in the experiments were observed during the moulting process. Initiation of the moulting process was taken to be the point when the old and new exuviae could be clearly recognised, and the end by the complete casting of the old exuviae (see *frontispiece*). Once the process had begun, the copepods were relatively inert, they would lie in the petri dish, and at regular intervals the body would convulse, slowly dislodging the exuviate. From the initiation to the completion of the process took about five minutes, and approximately 3% of individuals were observed to remain entangled in the old exuviae. The behaviour of the two species was also observed during the incubations. *Rhincalanus gigas* was relatively inactive: they hung upside down in the water and periodically wiped their antennae on their mouth parts. *Calanoides acutus* in comparison was quite active, and frequently displayed a jerky, seemingly random movement.

Stage durations did not differ systematically with date (Fig. 4.2). The moulting rate and number of individuals used in each determination, identified by station and year are summarised in Appendix III. Stage duration was generally short in the younger copepodite stages of both species. *Rhincalanus gigas* generally had slightly longer durations than similar stages of *Calanoides acutus*. The total number of individuals of each species stage that were incubated in moulting rate experiments, and the total number moulting, were then used to calculate a mean stage duration, these are summarised in Table 4.1. Development from stage CI - CIII took approximately two months in *R. gigas* while *C. acutus* took approximately one month to develop from stage CI - CIV.

Stage duration is shown in relation to mean water temperature in the top 60 m (Fig. 4.3), chlorophyll *a* concentration (Fig. 4.4) and silicate concentration (Fig. 4.5). Over the ambient temperature range of about 2°C experienced in this study, stage duration was not systematically related to temperature with the exception of *Calanoides acutus* stage CIV (r^2 adj. = -0.12, p = 0.025, Fig. 4.3). Similarly, stage duration was not systematically related to chlorophyll *a* concentration, except in *C. acutus* CIV (Fig. 4.4), or silicate concentration, except in *R. gigas* stage CIII (Fig. 4.5).

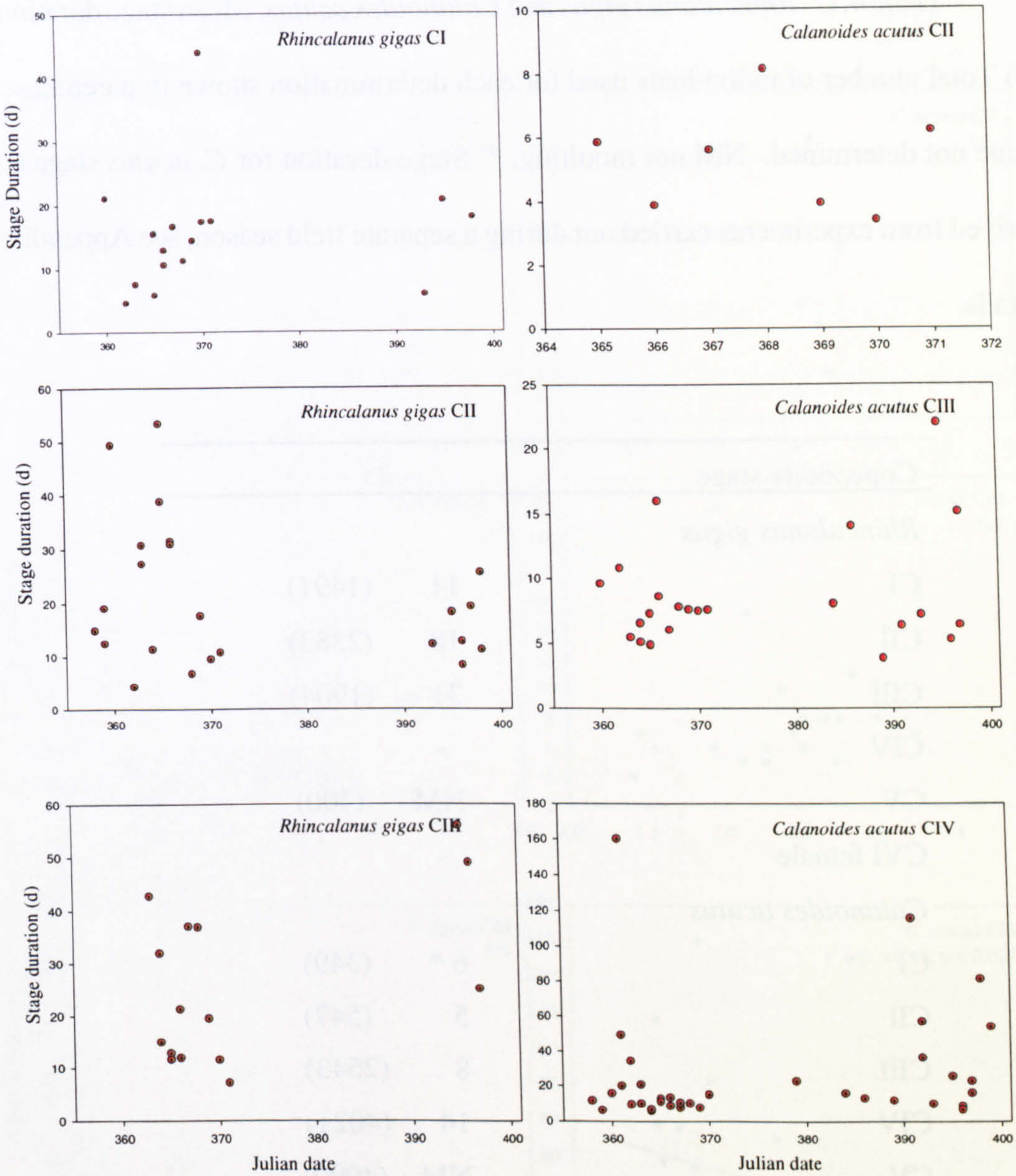


Figure 4.2 *Rhincalanus gigas* and *Calanoides acutus*. Stage duration (d) (●) plotted against Julian date (dates in January and February have 365 days added to make the comparison easier between late one year and early the next). Note the different scales on the y axis to clearly display the stage durations.

Table 4.1 *Rhincalanus gigas* and *Calanoides acutus*. Mean stage durations (D) (d) Total number of individuals used for each determination shown in parentheses. - : value not determined. NM not moulting. * Stage duration for *C. acutus* stage CI was derived from experiments carried out during a separate field season, see Appendix II for details.

Copepodite stage	D	
Rhincalanus gigas		
CI	11	(1491)
CII	18	(2383)
CIII	31	(1904)
CIV	-	
CV	NM	(400)
CVI female	-	
Calanoides acutus		
CI	6 *	(349)
CII	5	(547)
CIII	8	(2543)
CIV	14	(4021)
CV	NM	(4000)
CVI female	-	

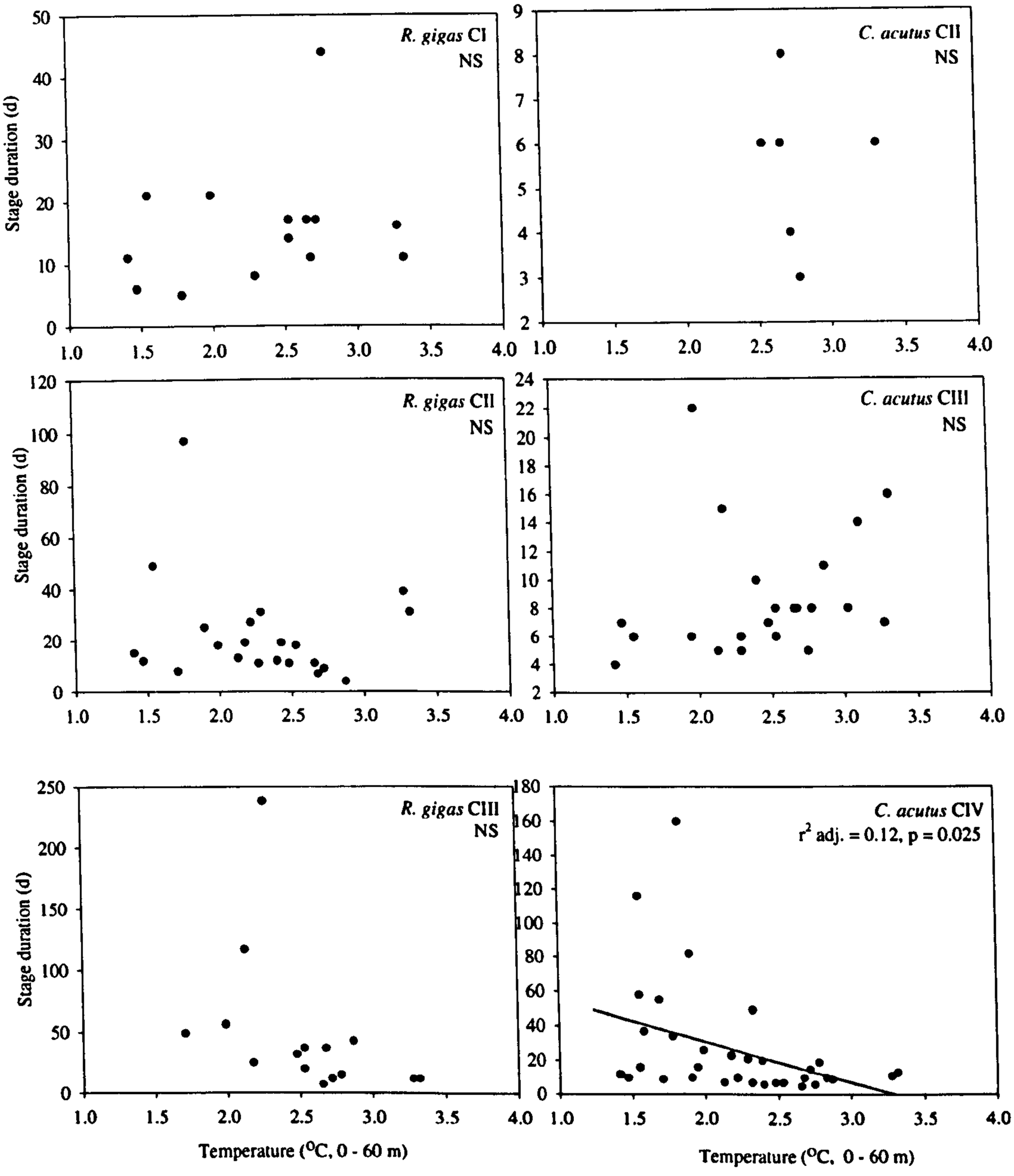


Figure 4.3 *Rhincalanus gigas* and *Calanoides acutus*. Stage duration in relation to mean temperature (°C) in the top 60 m. Least squares linear regression line fitted.

NS not significant.

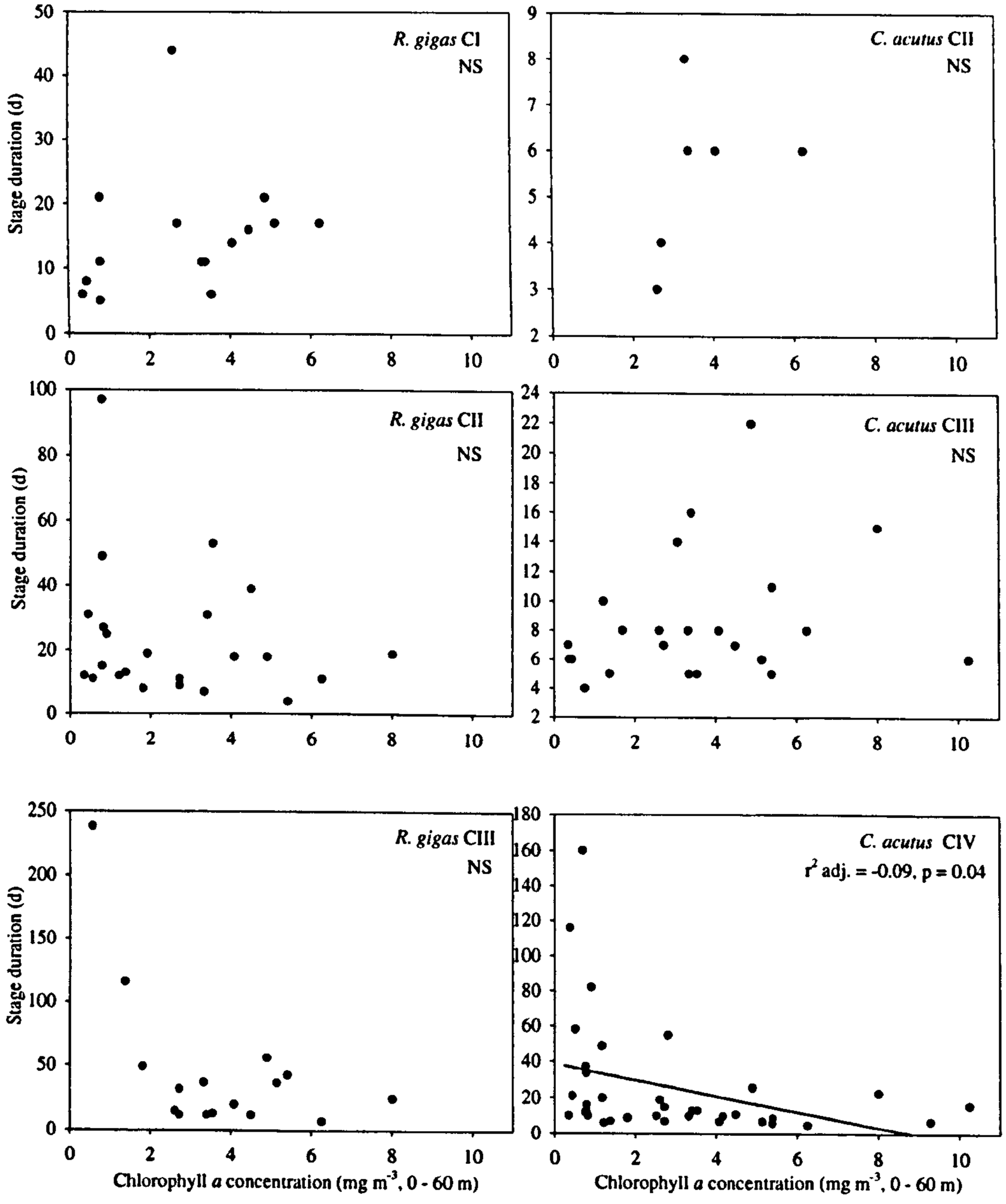


Figure 4.4 *Rhincalanus gigas* and *Calanoides acutus*. Stage duration in relation to integrated chlorophyll *a* (0 - 60 m). Least squares linear regression line fitted. NS not significant.

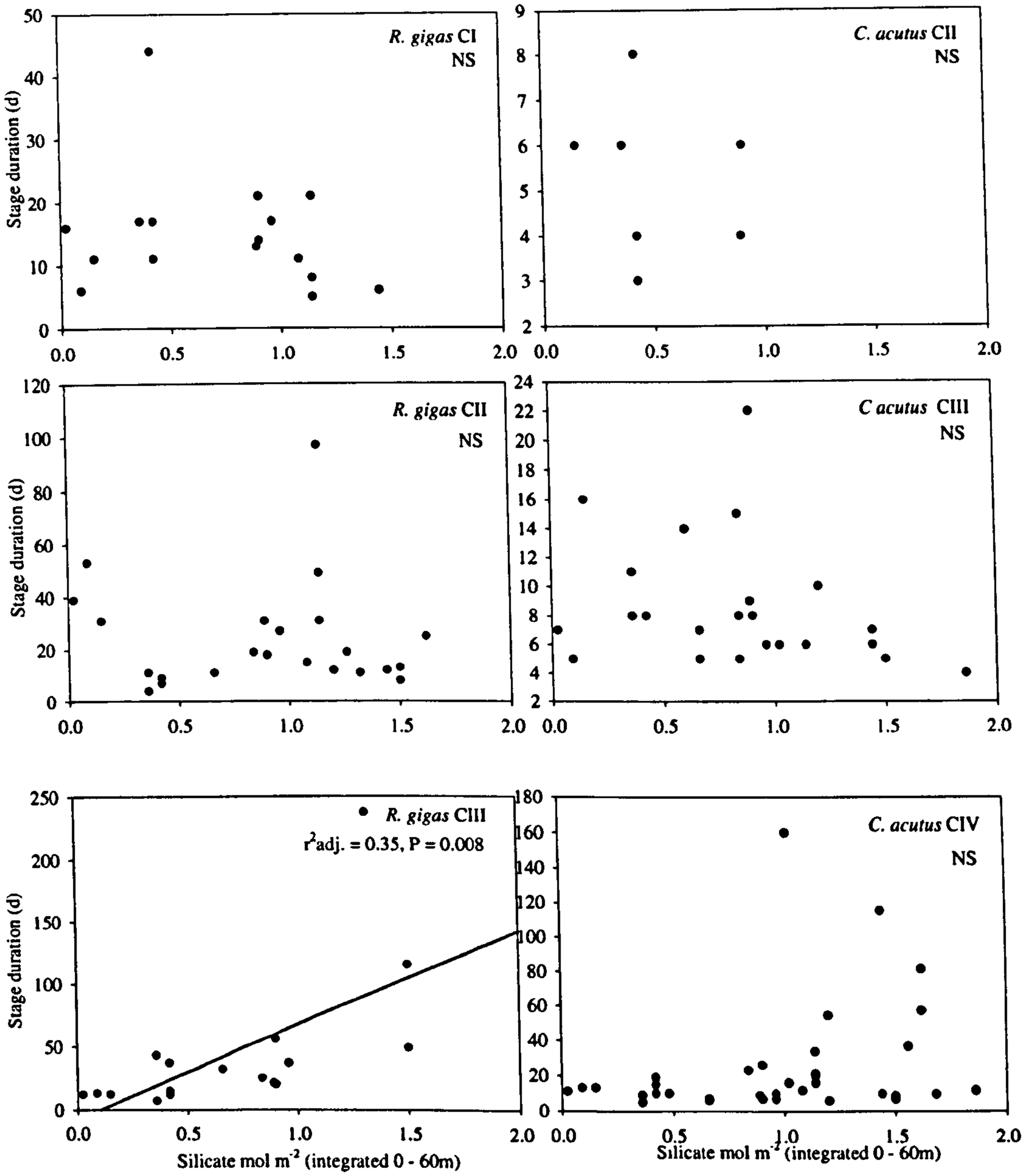


Figure 4.5 *Rhincalanus gigas* and *Calanoides acutus*. Stage duration in relation to integrated silicate (mol m^{-2}) (0 - 60 m). Least squares linear regression line fitted. NS not significant.

4.4 Discussion

Methodology

The method employed in this study is sensitive to the age within stage distribution of the species studied. Protracted spawning in these species has been reported by Marin (1988) and Atkinson (1991) which would suggest that age within stage is often normally distributed. However in populations where recruitment has just commenced there may be insufficient individuals within a stage old enough to moult to give an unbiassed estimate. This is unlikely to have been the case for *Calanoides acutus* given that recruitment commences as early as October (Andrews 1966) and the stage durations in this study were short. However it is possible that for *Rhincalanus gigas* such biases were present. Ward et al. (1997) indicated that the appearance of stage CI in the South Georgia region takes place in November at the earliest. Given the stage durations reported here for a period corresponding to some 4 - 6 weeks later, it is possible that stage CIII in particular may have unbalanced age within stage distributions i.e. insufficient animals old enough to moult. However, experiments conducted in this study only used stages which were abundant in the plankton, and it is suggested that this may overcome any major bias in stage duration being introduced to this data set.

Comparisons with other studies

Stage duration was relatively short in the younger copepodite stages of both *Calanoides acutus* and *Rhincalanus gigas*, with the development of *C. acutus* stages CI - CIV taking about one month and *R. gigas* stages CI - CIII taking about two months. Comparable estimates of development rates have been reported in a northern counterpart species *Calanus glacialis* during an incubation at 3°C, it developed from stage CI - CIV

in about one month (calculated from Corkett et al. 1986, their Fig. 3). Development rates measured in the current experiments may be compared with those reported in the literature which were derived from population stage frequency data. Rapid moulting in the younger stages of *Calanoides acutus* was suggested by the data of Andrews (1966), Atkinson (1991) and Atkinson et al. (1997), who estimated that the development from CI - CIII took about one month. Their estimates were based on the progression of the stage frequency of the population over time in the Antarctic Circumpolar Current. Andrews (1966) observed a mass recruitment of stage CIII individuals in the population during December, previous to which they had not been present. He was unable to make precise estimates of the rate of development of each stage because of the protracted length of the season during which early growth occurred, but concluded that early growth was quite rapid. This is in contrast to Huntley & Escritor (1991) who estimated that development of the copepodite stages took approximately one month per stage in the Gerlache and Bransfield Straits. They thought it improbable that the development of all naupliar stages could take place within one month, and yet if we assume that naupliar development would be in the same order as those observed in copepodite stage I in this study, then total naupliar development may well be in the order of one month. This would support the observations by Andrews (1966) that there is intense spawning activity in *C. acutus* during November and December giving rise to the abundance of early copepodite stages in late November/early December.

The cohort progression in the population of *Rhincalanus gigas* is less clear and there is little documented about the development time of the younger copepodite stages of this species. Nevertheless Ward et al. (1997) suggest rapid succession of copepodite stages in the more northerly parts of the range of this species and estimated that the

development time from stages CI - CIV was approximately two months. A similar duration was estimated in this study by summing the stage durations for the same range of copepodite stages which indicated a development time of around 62 d. Moulting rates measured in this study are therefore comparable with the fastest estimates derived from stage frequency data.

An estimate of the development time of these two species at the thermal extremes of their range can be made by using Bělehrádek's function in conjunction with experimentally determined stage durations of older copepodite stages made in this study. Bělehrádek's function ($D = a(T-\alpha)^{\wedge b}$) relates embryonic duration (D) to temperature (T). Three parameters; a (slope for the response), α (temperature scale position of the response) and b (curvature) were determined for the embryonic duration of *Rhincalanus gigas* and *Calanoides acutus* (Ward & Shreeve 1998). Application of the parameter values of a, α and b determined by Ward & Shreeve (1998) in the following equation:

$$D_0 = D_1\left[\frac{a(T_0 - \alpha)^{\wedge b}}{a(T_1 - \alpha)^{\wedge b}}\right]$$

allow the calculation of development time of stage i (D_0) at temperature T_0, from a known development time of stage i (D_1) at temperature T_1. Applying this to the development times of stages CI - CIII of *Rhincalanus gigas* measured at a mean of 2.2°C in this study, to a temperature which is more applicable to the more southerly extents of their range (0°C), increases the development from 60 to over 87 days. A similar application to stages CI - CIV of *Calanoides acutus* increases the duration from 32 to 38 days from 2.2 - 0°C.

This suggests that *R. gigas* takes much longer to develop at colder temperatures than does *C. acutus*. This offers some support for the extended two year life cycle proposed for *R. gigas* at the southern extents of its range (Ward et al. 1997 and references therein). This method assumes development of the early copepodite stages is equiproportional, whilst this does not appear to be the mode of development for the older stages, it is anticipated that is will give a reasonable estimate of the development of the younger stages at lower temperatures.

The development times estimated for the younger copepodite stages compared to stage CV in both *Rhincalanus gigas* and *Calanoides acutus*, tends to suggest a sigmoidal pattern of development. Stage CV of neither species moulted during the current survey. Similarly during a late spring survey in the South Georgia area, moulting in stage CV did not occur in *C. acutus* and was only recorded at one station for *R. gigas*, where a stage duration of 186 days was estimated (Ward & Shreeve 1999). This suggests that both species spend a large proportion of their life cycle in the older copepodite stages, this may well reflect higher mortality rates experienced in younger copepodite stages, as suggested for *Calanus marshallae* by Peterson (1986).

In Chapter 5 the carbon mass of the individuals in relation to temperature and food will be investigated, both for newly moulted individuals and for those that have been within the stage for at least 48 hours. This will facilitate the calculation of mass specific growth rates for individual stages, and allow investigation of suggestions that, within a stage, a critical mass may initiate moulting and that this may increase as food concentration increases (Carlotti & Sciandra 1989). Stage durations will also be used in

subsequent chapters, in conjunction with stage frequency data of the populations of *Rhincalanus gigas* and *Calanoides acutus*, to estimate stage specific rates of mortality in the younger copepodite stages using the model of 'vertical life table approach to zooplankton mortality estimation' described by Aksnes & Ohman (1996).

Chapter 5 Elemental composition of the copepodite stages of *Rhincalanus gigas* and *Calanoides acutus*

5.1 Introduction

Living organisms are products of their environment, changes in which are reflected in their chemical compositions. The elements which make up the greatest proportion of the composition of copepods are carbon, nitrogen, hydrogen, oxygen and phosphorus. Of these, carbon content is the most commonly measured element as it may be used to estimate biomass and energy content. Carbon is often reported as a proportion of the dry mass which may change with season and prevailing environmental conditions. The carbon to nitrogen ratio is frequently reported as it indicates the proportion of protein and lipid in the body composition. A low C:N mass ratio indicates a predominantly protein composition, whilst a higher C:N indicates a greater lipid component.

Throughout the worlds' oceans copepod carbon mass has been reported to range from c. 28 - 63% of their dry mass (Båmstedt 1986). Higher carbon contents have generally been associated with deep-living copepods or those found at higher latitudes. Conover and Huntley (1991) reviewed the carbon content of polar species of copepod and reported that for Antarctic species carbon mass was generally within the range of 39 - 49 % of body dry mass. These values were based on three studies which were conducted by Ikeda & Mitchell (1982), Schnack (1985) and Schnack et al. (1985), whom measured the carbon content in the older copepodite stages, CV and CVI of four biomass dominant

copepods; *Calanus propinquus*, *Calanoides acutus*, *Rhincalanus gigas* and *Metridia gerlachei*. Huntley and Nordhausen (1995) examined the older copepodite stages CV and CVI of *Rhincalanus gigas*, *Calanoides acutus* and *Metridia gerlachei* during the austral winter and reported carbon as a proportion of dry mass to range from 38 to 54 %. Literature values for carbon mass of Antarctic copepods is therefore biassed towards the older, lipid storing stages.

Temperature and food concentration are the key environmental parameters which influence copepod carbon mass. Their effects were investigated by Vidal (1980b) for two temperate species of copepod, *Calanus pacificus* and *Pseudocalanus sp.* cultured under various combinations of phytoplankton concentration and temperature. He concluded that mean dry mass of the early copepodite stages (CI - CIII) was relatively unaffected by either food concentration or temperature; but that in the older stages it increased hyperbolically with food concentration and was also inversely related to temperature. The Southern Ocean is characterised by the combination of low but relatively stable sea-water temperatures with a markedly seasonal pattern of primary production. The timing and intensity of the phytoplankton bloom may therefore ultimately be the major factor governing changes in copepod body mass in the Southern Ocean, whereas temperature, which is characterised by a low seasonal range, may have less of an effect.

In this study accurate determinations of the dry, carbon and nitrogen mass in a range of copepodite stages of *Rhincalanus gigas* and *Calanoides acutus*, of both newly moulted individuals and those which have been within the stage for at least 48 hours are presented. These carbon masses are used in Chapter 6, in conjunction with stage

durations determined in Chapter 4, to determine mass specific growth rates of each copepodite stage. The relationship of carbon mass to the prevailing environmental parameters of temperature and food concentration are determined. Total lipid mass is measured to facilitate comparison of total dry mass and structural mass between species stages, and to estimate mass specific growth in the non-moulting stage CV.

5.2 Materials and methods.

Body dry mass and the elemental composition of carbon and nitrogen was determined for copepods which had been used in the moulting rate experiments (see Chapter 4). Individuals were rinsed in 4% ammonium formate in distilled water, blotted dry and then placed in pre-weighed ultra light-weight tin foil capsules. To facilitate accurate measurement of dry mass, younger copepodite stages were pooled within stage as follows: 20 individual CII *Calanoides acutus*, 10 each of *C. acutus* stages CIII and CIV and *Rhincalanus gigas* stages CI and CII; 5 of *C. acutus* stages CV and CVI females and *R. gigas* stages CIII- CV; adult female *R. gigas* were treated individually. These samples were then frozen at - 80°C and subsequently dried at 60°C onboard ship within one week. Samples were transferred to the UK in a sealed container, where they were again dried at 60°C to a constant weight. Dry mass was measured on a Mettler MT5 balance to an accuracy of ± 1 μg. Whole samples were then analysed for carbon, hydrogen and nitrogen content using a Fisons EA 1108 elemental analyser. Calibration curves were constructed using the standard acetanilide. Individuals which had moulted during all the experiments were preserved and analysed separately. This allowed the comparison of carbon mass of newly moulted individuals with those that had been in stage for more than 48 hours.

It was considered a possibility that copepods may have lost carbon mass over the 48 hour incubation period, during which time they had been kept in filtered sea-water. This would consequently mean an under-estimation of mass specific growth rates in the field if significant bodily carbon mass was lost. To investigate this, individuals of the same species and stage, and from the same station, were either maintained in the 48 hr incubations or preserved immediately upon capture. Carbon mass of individuals was then compared to see if any significant loses had occurred.

Lipid mass was determined in individuals which had been used in moulting rate experiments. Batches of between 30 and 100 species stages were placed in vials containing 2:1 (v:v) chloroform:methanol solution. The vial was then flushed in a stream of nitrogen, to displace all oxygen within the vial, the lid was then secured and the whole sample frozen at -80°C. In the UK the total lipid fraction was extracted according to Folch et al. (1957) and Christie (1982). Samples were homogenised and the resulting chloroform lipid fraction filtered through a 0.45 μm pore PTFE filter. Samples were then transferred to pre-weighed tin capsules and dried to a constant weight under a stream of nitrogen. Lipid mass was measured on a Mettler MT5 balance to an accuracy of ± 1 μg.

Statistical analysis

Relationships between carbon mass and the environmental variables described in Chapter 3 were tested using either linear or curvilinear least squares regression. These two approaches were used because, although linear regression assumes the least about the data, it has also been suggested that the effect of food concentration on body mass may alter with developmental stage. For example Vidal (1980a) showed that for stage CII of

Calanus pacificus body mass was the same regardless of food concentration, whereas the mass of the latter stages increased hyperbolically with food concentration. The relationship which proved most significant is presented. All statistical relationships were explored using either Minitab ® version 13 or Sigmaplot ® version 7.

5.3 Results

Carbon loss during the experiments

There was no significant difference in carbon mass of individuals that were either incubated in filtered sea-water for 48 hours or that were preserved immediately upon capture. Four experiments were conducted where 2 lots of 30 individuals of *Rhincalanus gigas* CVI or *Calanoides acutus* stage CV were either incubated in filtered sea-water for 48 hours or preserved immediately. These were the only stages which were both sufficiently abundant and did not need bulking in large groups for carbon mass determinations. This allowed statistically significant comparisons of pre- and post-incubations to be made. Carbon mass was determined for each group and an ANOVA showed no significant difference between these two treatments, results are shown in Table 5.1. It is concluded that during the 48 hours of the incubation, individuals did not lose a significant proportion of their body carbon mass. Any subsequent estimates of the mass specific growth rates using these figures would therefore represent the growth in field conditions.

Table 5.1 Body carbon mass loss on incubation. Mean carbon mass (CM) ± standard deviation of individuals at initiation of experiments (T_0) and after 48 hours incubation (T_1). ANOVA results for four experiments conducted on *Rhincalanus gigas* and *Calanoides acutus*, showing F and p values.

Species stage	CM T_0	CM T_1	F value	p value
Rhincalanus gigas CVI f	2180 ± 461	2749 ± 1408	3.60	0.062
Rhincalanus gigas CVI f	1755 ± 434	1725 ± 390	0.03	0.870
Calanoides acutus CV	602 ± 184	469 ± 189	3.53	0.072
Calanoides acutus CV	348 ± 107	383 ± 272	0.18	0.677

Carbon mass of newly moulted individuals

Carbon mass of newly moulted individuals generally were between the heaviest of the previous stage and the lightest of the current one, although not all individuals within a stage always moulted at the top end of the range (Fig. 5.1). Thus newly moulted individuals did not represent a random spread of individual carbon masses with stage, but were generally restricted to the heaviest of the stage they had moulted from. The range of carbon mass figured in 5.1 are for the younger copepodite stages of *Rhincalanus gigas*, both of newly moulted individuals and those which did not moult during the experiments.

Carbon mass of newly moulted individuals was compared to three environmental variables; silicate concentration, which was used as a proxy for past feeding conditions (Chapter 3), chlorophyll *a* concentration and temperature. This was to investigate whether all individuals of a particular species stage would moult at the same mass, or if the mass

upon moulting would vary with changing food and temperature regimes. This investigation was only possible for *Calanoides acutus* stage CIV moulting to CV, as insufficient individuals were available to make this comparison for other stages. Newly moulted stage CV *C. acutus* displayed a wide range in carbon mass from 27 to 169 µg ind^{-1} (Fig. 5.2). There was a significant negative relationship between carbon mass of newly moulted stage CVs and silicate (r^2 adj. = 0.27, p = 0.003), showing that the mass upon moulting did increase in line with a recent higher concentrations of phytoplankton in the water column (Fig. 5.3). There was no significant relationship between chlorophyll *a* and carbon mass of newly moulted stage CVs, but a strong positive relationship was found with temperature (r^2 adj. = 0.38, p < 0.0001) (Fig. 5.3).

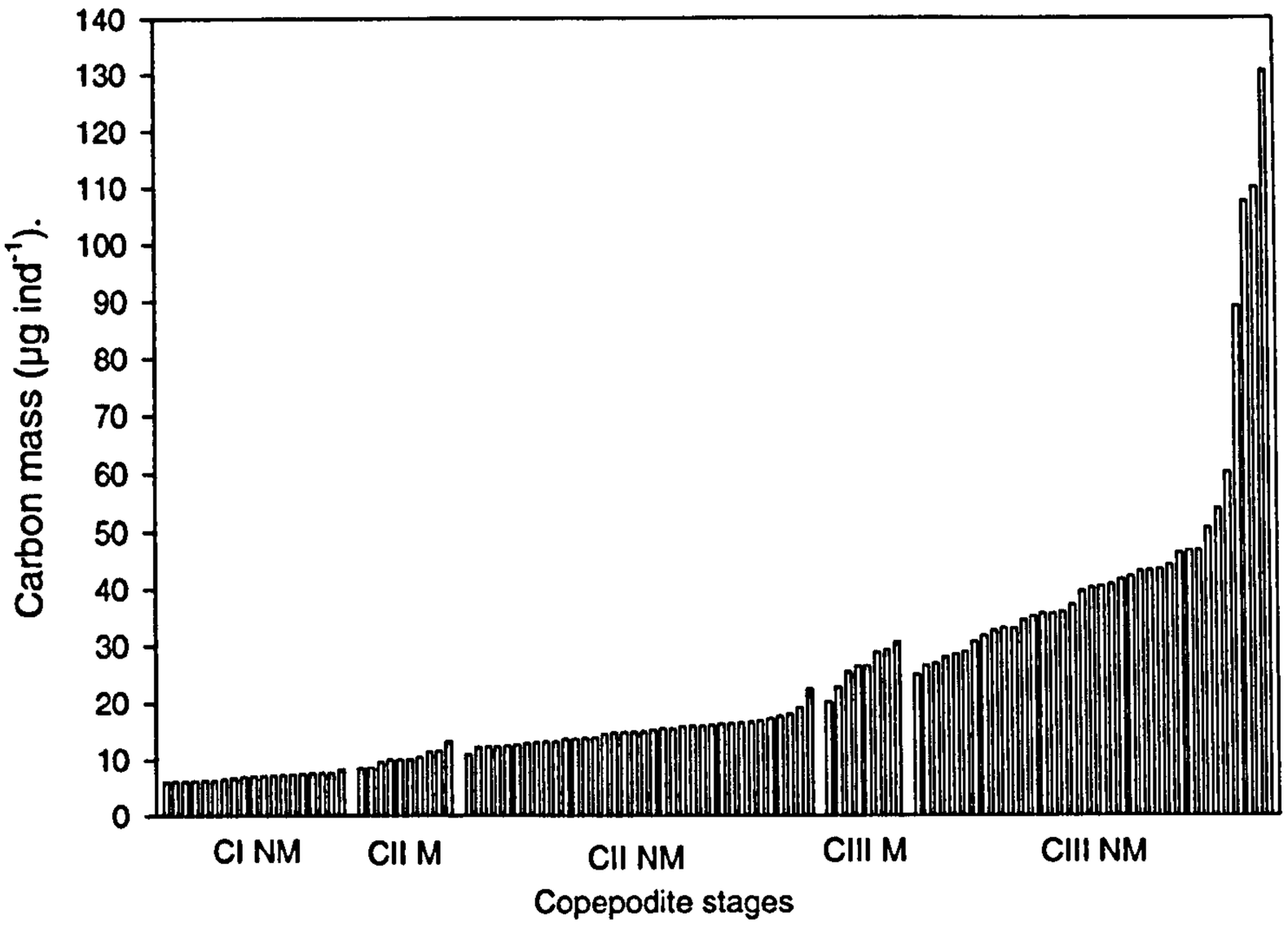


Figure 5.1 *Rhincalanus gigas*. Ranked carbon mass (µg ind^{-1}) of batches of individuals in each copepodite stage CI - CIII. NM - individuals that did not moult during the experiments, M - individuals which did moult during the experiments. Data from 1996/97 season, off-shelf in the western mesoscale box, where sufficient individuals were available for comparison.

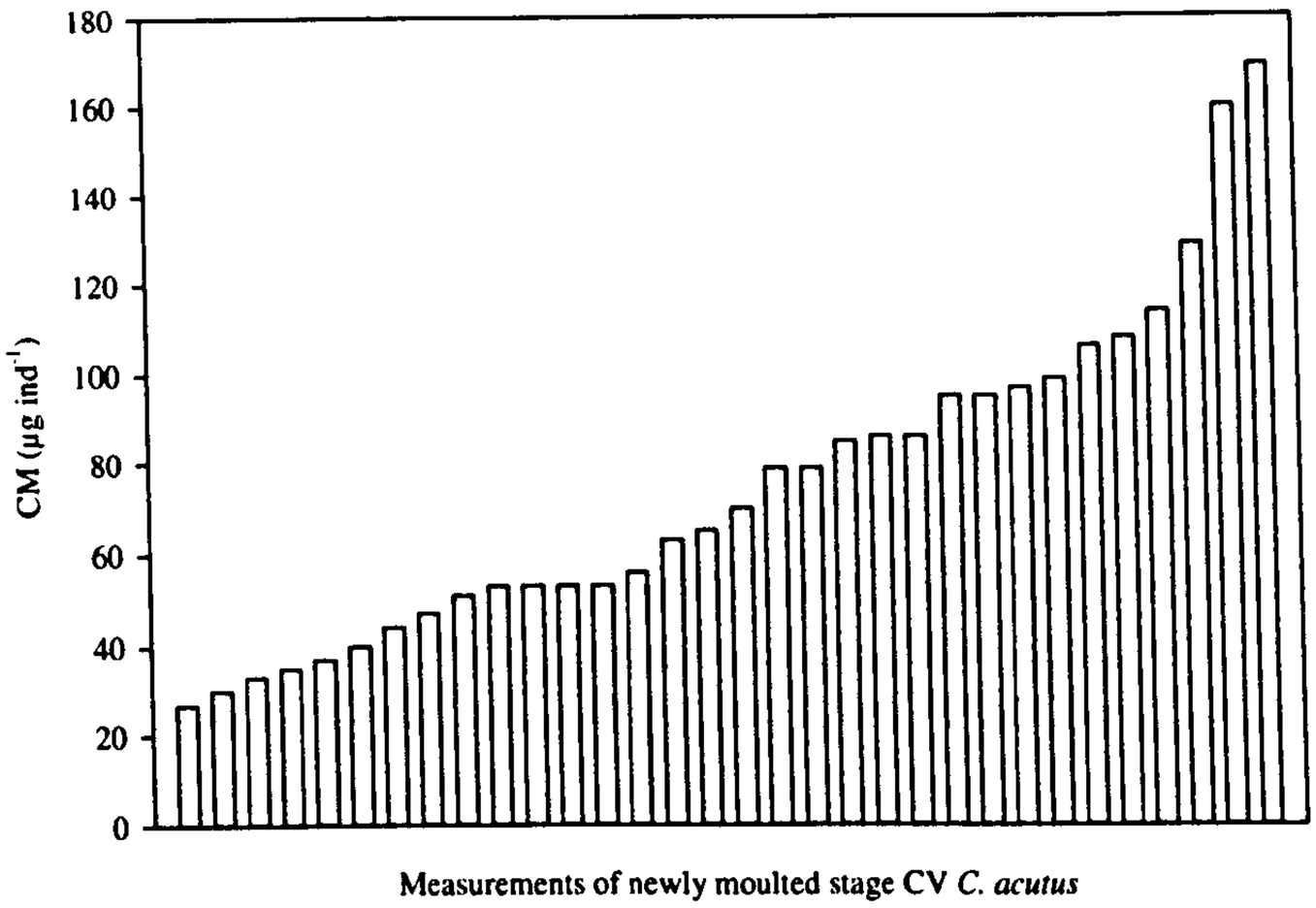


Figure 5.2 Carbon mass (CM µg ind^{-1}) of newly moulted *Calanoides acutus* stage CV. Each measurement is the mean of five individuals. Data ranked to clarify the distribution of carbon mass within the stage.

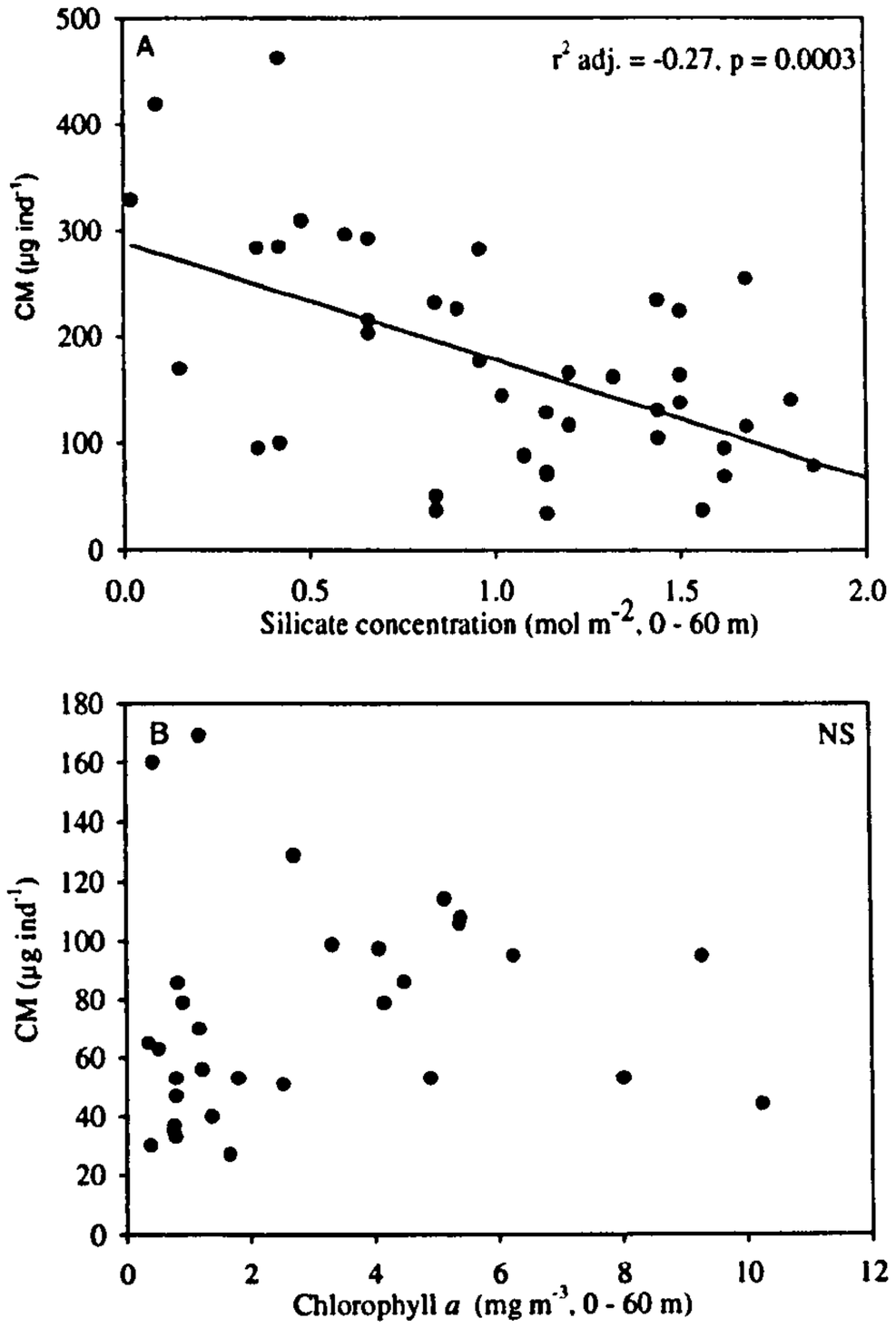


Figure 5.3 *Calanoides acutus* stage CV. Carbon mass (CM) of newly moulted individuals in relation to A. silicate concentration (mol m^{-2}, 0 - 60m), B. chlorophyll *a* (mg m^{-3}, 0 - 60 m) and C. temperature (°C, 0 - 60m).

Carbon mass, dry mass, C:N ratio and lipid mass.

The range and distribution of carbon mass for each species stage, representing all stations and years, is shown in Fig. 5.4. Carbon mass was highly variable over the study period, with the greatest range seen in the older lipid storing stages of both species. Mean carbon mass (μg C ind^{-1}) of *Rhincalanus gigas* ranged from 6 μg ind^{-1} in CI to 1037 μg in CVI females. Expressed in terms of the proportion of dry mass, this increased from 15 - 52 % from stage CI - CVI females respectively. Mean carbon mass (μg C ind^{-1}) of *Calanoides acutus* ranged from 4 μg ind^{-1} in CII to 463 μg in CVI females. This expressed in terms of the proportion of dry mass represents increases from 20 - 59 % from stage CII - CVI females respectively. Mean carbon mass and the range for each species and stage and its proportion of dry mass are given in Table 5.2.

Carbon to nitrogen mass ratio was low in the younger copepodite stages of both *Rhincalanus gigas* and *Calanoides acutus*, with a mean value of about 3 for stages CI - CIII (Table 5.2). This increased to a mean of 6.5 in the older lipid storing stages CV - CVI female.

Lipid mass is shown as the difference between the dry and structural mass (Fig. 5.5). The proportion of lipid increases in the older copepodite stages. The younger copepodite stages of *Rhincalanus gigas* have very low lipid content, whereas in stages CV and CVI f lipid makes up between 16 and 28 % of dry mass respectively. In *Calanoides acutus* stages CV and CVI f lipid content varies between 37 and 15 % respectively.

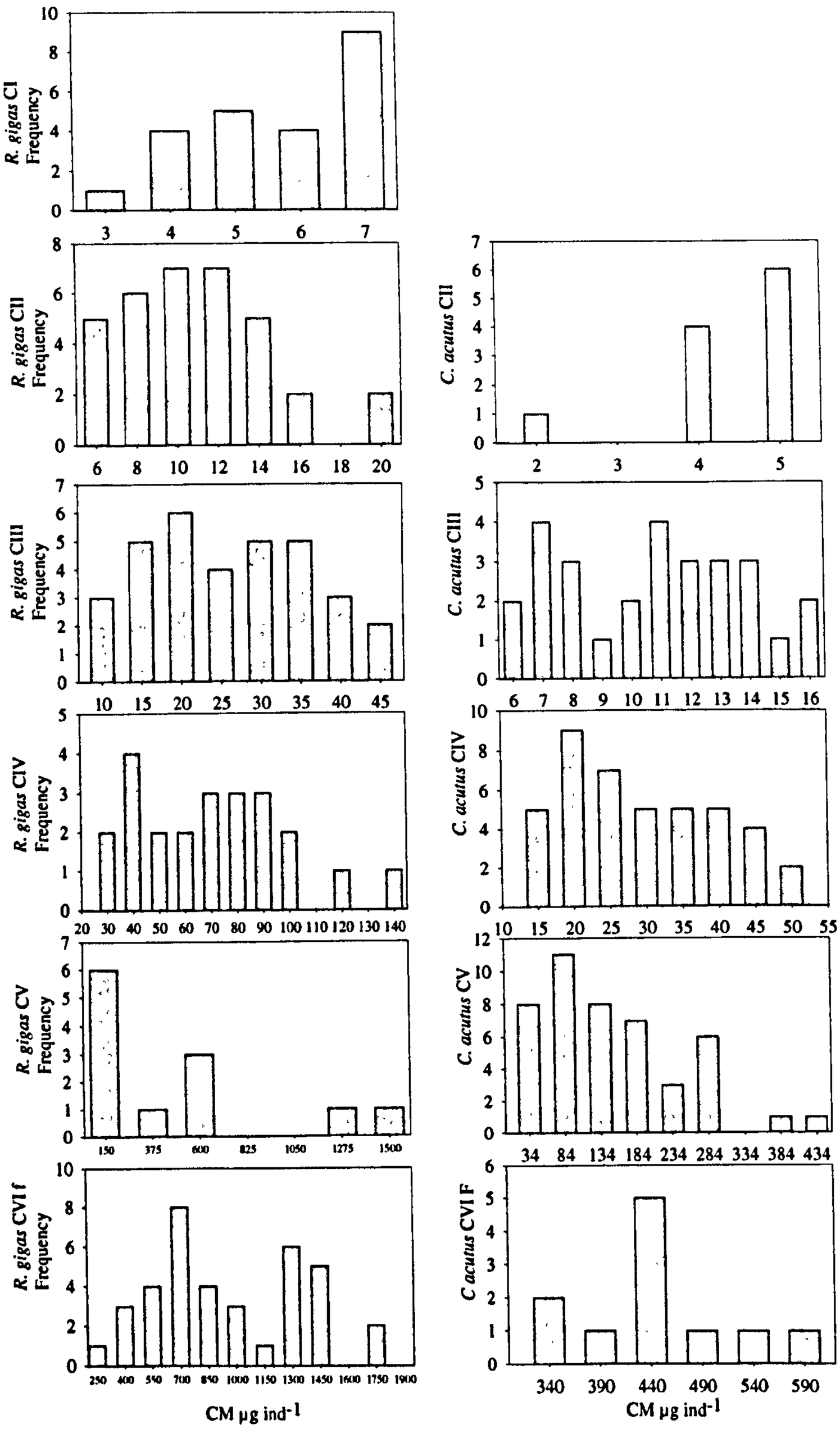
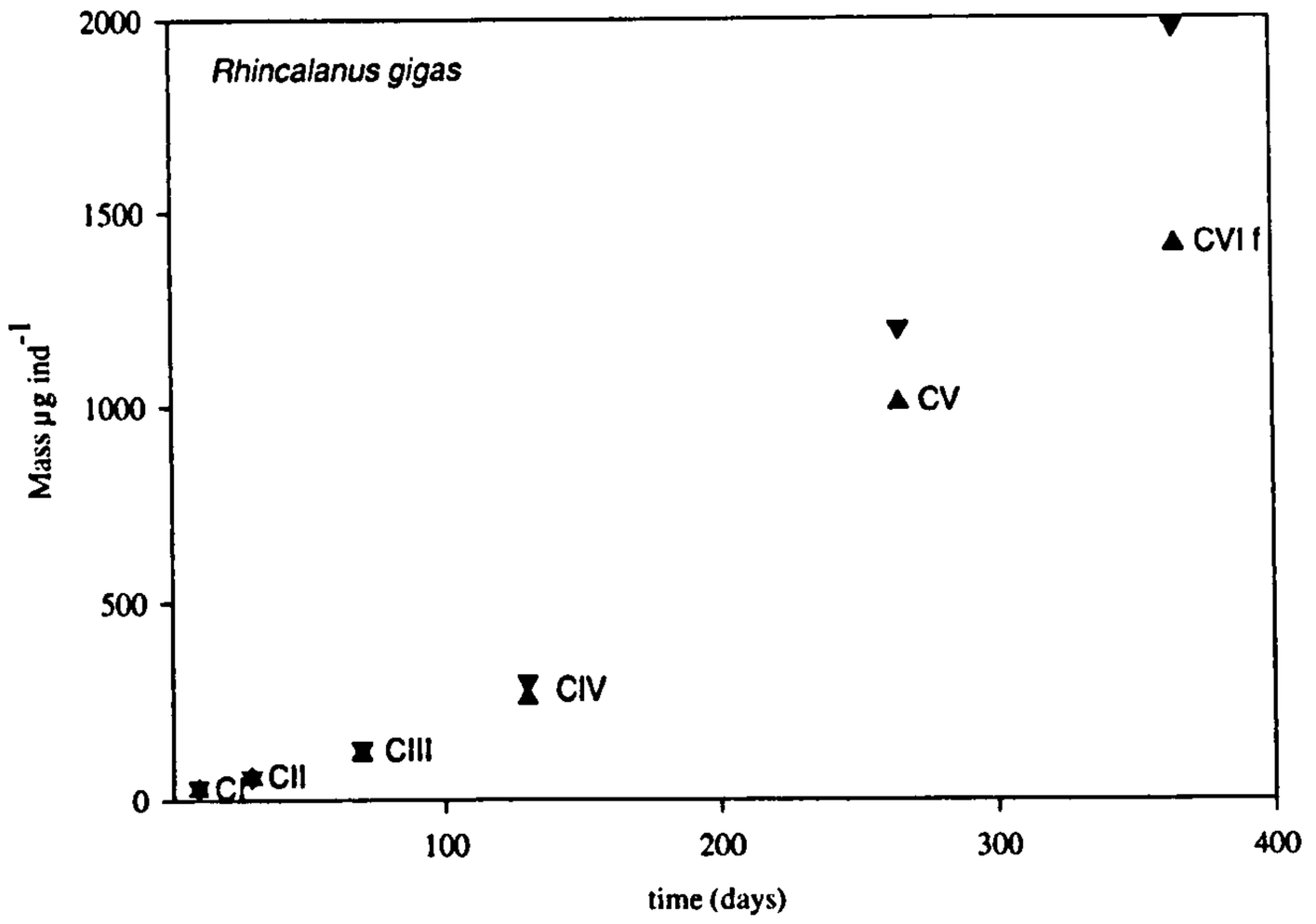


Figure 5.4 *Rhincalanus gigas* and *Calanoides acutus*. Range and distribution of carbon mass (CM, µg ind^{-1}), for each stage, all stations and years included.

Table 5.2. *Rhincalanus gigas* and *Calanoides acutus*. Mean carbon mass (CM μg ind^{-1}) with range between batches in parenthesis, carbon mass as a percentage of dry mass (CM % DM) and carbon to nitrogen mass ratio (C:N), range shown in parentheses, and lipid mass (LM, μg ind^{-1}). Numbers of individuals used in each determination, n. No data -.

Stage	CM	CM % DM	C:N	LM	n
Rhincalanus gigas					
CI	6 (3-7)	15	2.97 (2.28 - 4.19)	7	830
CII	11 (6-21)	19	2.90 (2.29 - 3.80)	5	2117
CIII	28 (11-45)	25	3.12 (2.46 - 4.53)	15	1466
CIV	75 (33-145)	26	3.81 (2.37 - 4.99)	38	472
CV	560 (159-1506)	51	5.09 (3.30 - 8.90)	193	671
CVI fem	1037 (288-1791)	52	6.38 (4.00 - 8.40)	568	691
Calanoides acutus					
CII	4 (2-5)	20	3.43 (3.20 - 3.89)	-	281
CIII	11 (6-16)	23	3.66 (2.69 - 3.90)	13	1396
CIV	31 (15-54)	29	3.87 (2.60 - 5.98)	12	2537
CV	176 (34-462)	46	6.32 (2.95 - 9.71)	142	2443
CVI fem	463 (343-599)	59	8.20 (5.70 - 9.40)	120	368



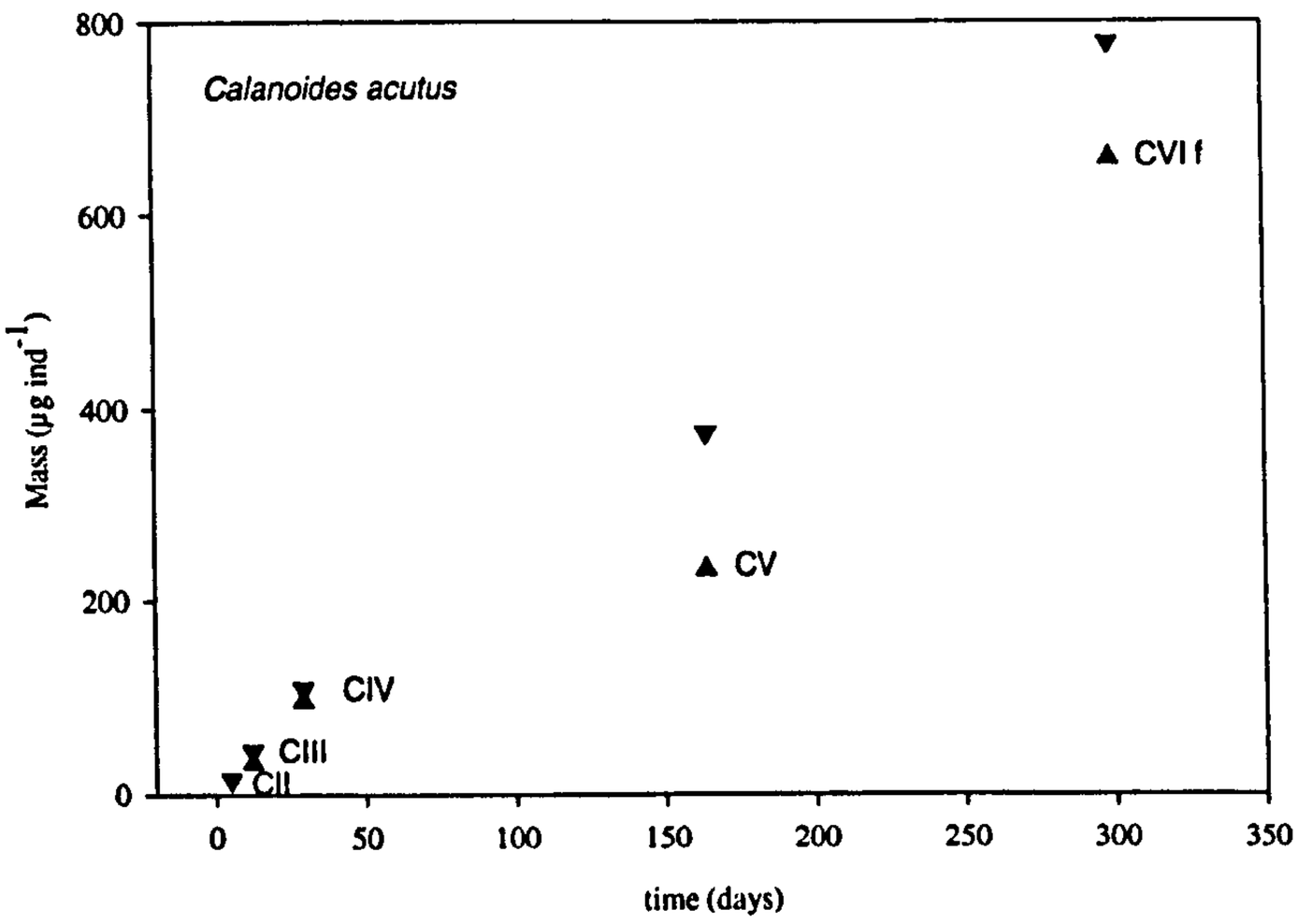


Figure 5.5 *Rhincalanus gigas* and *Calanoides acutus*. Individual dry mass (µg ind^{-1}) (▼)and structural mass (µg ind^{-1}) (▲) for each stage, plotted against time in stage. Stage duration for *R. gigas* stages CI - CIII and *C. acutus* CII- CIV measured during this study, duration for other stages estimated from stage frequency data of Ward et al. (1997) and Atkinson et al. (1997) for *R. gigas* and *C. acutus* respectively. Structural mass is defined here as dry mass (µg ind^{-1}) minus the lipid mass.

Carbon mass in relation to environmental variables.

Silicate Silicate depletion was used as a proxy of the copepods past feeding environment (Chapter 3). Least squares linear regression best described the relationship between silicate concentration and individual carbon mass. A depletion of silicate concentration from 2 down to 0 mol m^{-2} (0 - 60 m), was associated with a doubling of body carbon mass in individuals in 8 out of 11 tests, (Fig. 5.6 and Table 5.3).

Chlorophyll a A curvilinear regression best described the relationship between body carbon mass and chlorophyll *a* concentration, giving significant regressions in 7 out of 11 tests. An exponential increase in carbon mass occurred between approximately 0 and 3 mg chlorophyll *a* m^{-3} (0 - 60m), at higher concentrations carbon mass remained relatively constant (Fig. 5.7). Parameter values for the fitted non-linear regression line, $y = a(1- \exp(-b*x))$, for each species and stage are defined in Table 5.4. Adjusted r^2 and p values are presented in Table 5.3.

Temperature Carbon mass was related positively to temperature in 8 out of 11 tests, and best fits were achieved assuming a linear relationship (Fig. 5.8 and Table 5.3). An increase in water temperature from around 1.5 to 3.5°C was generally associated with a doubling of the carbon mass of individuals.

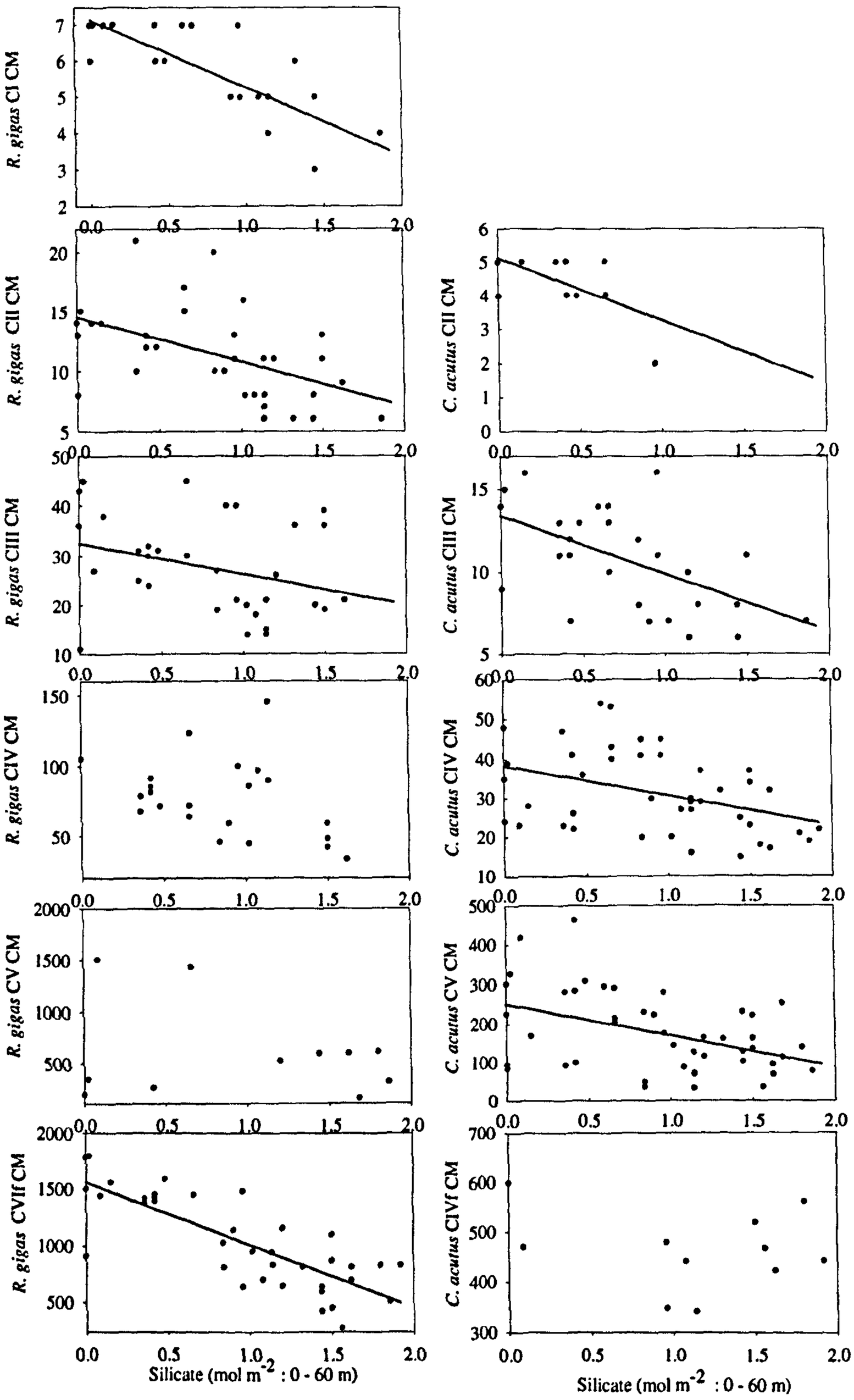


Figure 5.6 *Rhincalanus gigas* and *Calanoides acutus*. Carbon mass in relation to silicate concentration. Least squares linear regression lines fitted. See Table 5.3 for values of r^2 adj. and p values.

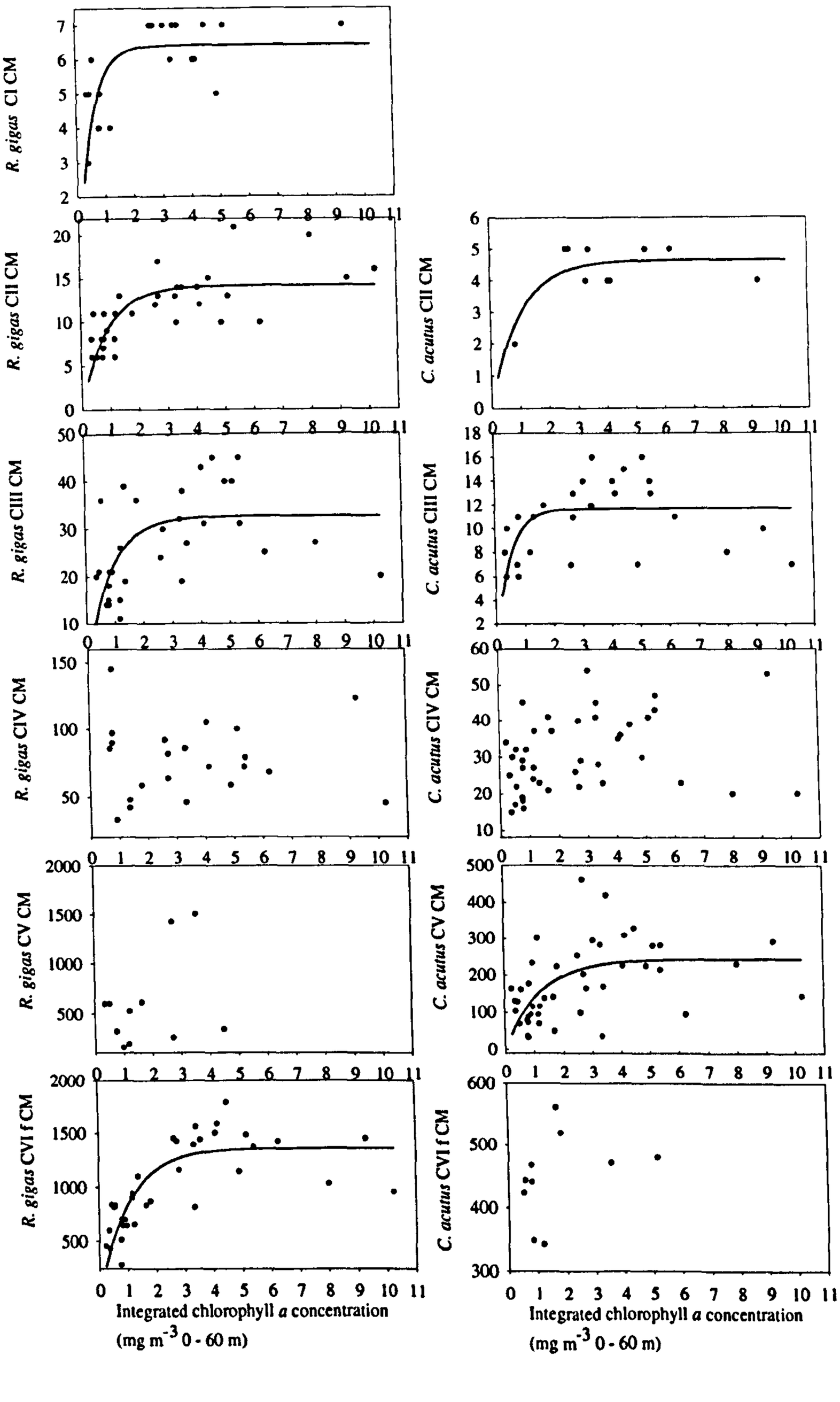


Figure 5.7 *Rhincalanus gigas* and *Calanoides acutus*. Carbon mass (CM) in relation to Chlorophyll *a* concentration. Regression curve fitted y = a (1-exp(-b*x). Parameter values a and b defined in Table 5.4 and r^2 adj. and p values in Table 5.3.

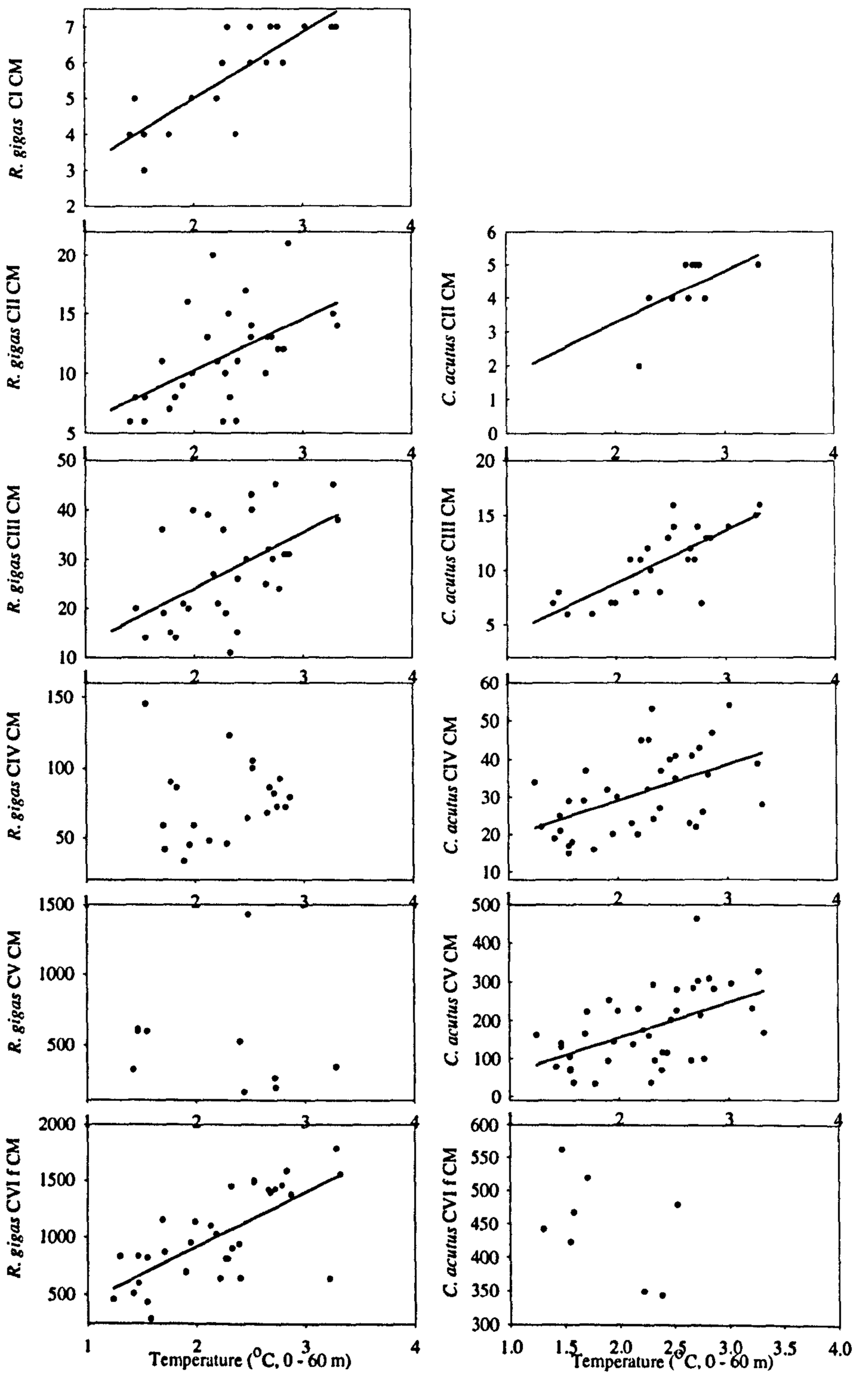


Figure 5.8 *Rhincalanus gigas* and *Calanoides acutus*. Carbon mass (CM) in relation to temperature. Least squares linear regression fitted. See Table 5.3 for r^2 adj. values and p values.

Table 5.3 *Rhincalanus gigas* and *Calanoides acutus*. Regression results for Figs. 5.6, 5.7 & 5.8. Carbon mass in relation to, Silicate (mol m^{-2} 0 - 60 m) (Si), Chlorophyll *a* (mg m^{-3}) (Chl) and temperature (°C) (T) * $p < 0.05$, ** = $p < 0.0001$. NS not significant. Significant values shown in bold.

Stage	r^2 adj (Si)	r^2 adj (Chl)	r^2 adj (T)
Rhincalanus gigas			
CI	**0.60 ****	**0.41 ****	**0.65 ****
CII	**0.33 ****	**0.48 ****	**0.26 ***
CIII	**0.09 ***	**0.24 ****	**0.26 ***
CIV	NS	NS	NS
CV	NS	NS	NS
CVI female	**0.67 ****	**0.62 ****	**0.48****
Calanoides acutus			
CII	**0.54 ****	**0.57 ***	**0.39 ***
CIII	**0.34 ****	**0.19 ****	**0.58 ****
CIV	**0.15 ***	NS	**0.25 ***
CV	**0.27 ****	**0.26 ***	**0.27 ***
CVI female	NS	NS	NS

Table 5.4 *Rhincalanus gigas* and *Calanoides acutus*. Carbon mass in relation to chlorophyll *a*. Coefficient values a and b defined for Fig. 5.7. Curvilinear regression line fitted y = a(1-exp(-b*x).

Stage	a	b
Rhincalanus gigas		
CI	6.43	1.99
CII	14.20	1.10
CIII	32.61	1.19
CIV	-	-
CV	-	-
CVI female	1361.00	0.95
Calanoides acutus		
CII	4.64	0.98
CIII	11.70	1.97
CIV	-	-
CV	244.00	0.81
CVI female	-	-

5.4 Discussion.

Carbon mass of individuals within a stage was variable and was negatively related to the concentration of silicate, and positively related to chlorophyll *a* and temperature. Although carbon mass gave a positive significant relationship with temperature, this contrasts with the findings of Vidal (1980a). He observed that at colder temperatures, individuals will put on more carbon mass within a stage, due to them remaining in stage for longer. However, having demonstrated in this study (Chapter 4) that there are no systematic differences in stage duration within each stage in relation to temperature, we conclude that food, rather than temperature, was the major factor explaining variation in carbon mass during the course of our study, but that temperature may influence carbon mass indirectly.

In Chapter 3 (Fig. 3.13) it was demonstrated that temperature was positively correlated with standing stock of chlorophyll *a,* and that an increase of 1°C in the mean sea-water temperature (0 - 60m) could be related to a doubling in the standing stock of chlorophyll *a*. The critical concentration of chlorophyll *a* for each species and stage to attain maximum carbon mass observed in this study was about 3 mg chl m^{-3} (integrated over the top 60 m). Below this concentration carbon mass fell sharply, whereas above this critical level carbon mass did not increase significantly (see Fig 5.7). This concentration of chlorophyll *a* was coincident with water temperatures of about 2.75°C and above (refer to Chapter 3 Fig. 3.13). Copepod carbon mass measured in this study therefore seems to be affected primarily by food concentration, which in turn appears to be associated with prevailing temperatures.

Comparisons with other studies

The mean proportion of carbon to dry mass of 51% for stages CV and CVI *Rhincalanus gigas* measured in this study is slightly higher than the 44% reported by Schnack et al. (1985). Their study was however carried out both at a higher latitude, and earlier in the season, during November and December, whilst this study was carried out predominantly during December and January. Due to the timing of our study the copepods may have been exposed to the summer phytoplankton bloom for longer. This in turn would have allowed these older copepodite stages to increase their lipid stores and in so doing increase the amount of carbon as a proportion of dry mass (Hagen & Schnack -Schiel 1996). Indeed the carbon to nitrogen mass ratio (C:N) for *Rhincalanus gigas* CV and CVI females was higher in this study, with a mean of 5.7 compared to only 4.0 reported by Schnack et al. (1985). The higher C:N ratio is indicative of proportionally larger lipid reserves, whilst a predominantly protein composition is reflected by a lower C:N ratio. The pattern observed for *R. gigas* was also observed for *Calanoides acutus*. In this study the mean proportion of carbon to dry mass in stages CV and CVI *C. acutus* was 53 % with a C:N of 7.26, compared to only 44 % and a C:N of 4 reported by Schnack et al. (1985).

Carbon mass has not been reported previously for the younger copepodite stages of *Rhincalanus gigas* or *Calanoides acutus*, indeed there appears to be little data for the younger copepodite stages of any marine species of *Calanus* from any location. Ikeda (1974) and Båmstedt (1986 and references therein) reported carbon contents of marine copepods from different parts of the worlds oceans to range from *c* 20 - 63% of dry mass, with higher values associated with higher latitudes. Whilst their study was dominated by older copepodite stages, Bottrell & Robins (1984) reported a seasonal range of 35 - 50%

for *Calanus helgolandicus* stages CIII. The carbon contents presented in this study therefore fall within the reported range, but are much lower than has been assumed in other studies for younger copepodite stages of Antarctic copepods. Consequently any assumption that younger copepodite stages have a similar proportion of carbon to dry mass as older stages, would lead to under estimates of some rate processes. For example Atkinson (1994) assumed body carbon was 45% of dry mass when estimating the proportion of body carbon ingested by younger copepodite stages, his estimates would double if the lower proportion of carbon to dry mass found in this study were used.

Mass on moulting

Carbon mass of newly moulted individuals of both species generally lay at the upper end of the range of the previous stage and the lower end of the current one (Fig. 5.1). Thus newly moulted individuals did not represent a random spread of individual carbon mass within a stage but were restricted to the heaviest individuals, suggesting as Miller et al. (1984) proposed for *Calanus pacificus*, that a threshold is reached beyond which moulting will occur. This suggests that the copepods used in these moulting rate experiments would have naturally moulted within the 48 hour incubation period and have not been induced to do so by handling procedures.

Although the carbon mass was within a narrow band within a station, when compared to silicate concentration across all years and stations there was a significant negative relationship. This suggests that the mass upon moulting was a reflection of the past feeding history, and that there was not an endogenous critical mass that the individuals of a particular stage must reach before initiation of moulting. This

experimentally derived data supports the theoretical ideas used in models by Carlotti & Sciandra (1989) who suggested that there may be a critical moulting weight, and that this may shift towards higher values when the food concentration increases.

Lipid

Lipid forms a similar proportion of dry mass in *Calanoides acutus* stage CV to that reported by Hagen & Schnack-Schiel (1996) during austral summer. They reported lipid as a proportion of dry mass as ranging from 35 and 55% during late January/February, compared to mean measurements of about 37% made a month earlier (mid December/ Mid January) in this study. The values in this study therefore fall at the lower end of the range reported by Hagen & Schnack-Schiel (1996) and are most likely explained by the fact that lipid deposition has not progressed as far in individuals in the current study.

5.5 Conclusions

Carbon mass of each species stage was variable and appeared most strongly and negatively related to silicate concentration and chlorophyll *a*, an indication of the role of past and present phytoplankton production in determining body mass. Body carbon mass upon moulting was found to be within a limited range for individuals from the same sample, but it varied between samples and was also significantly negatively related to silicate concentration. Body carbon content (as a % of dry mass) increased with increasing stage of development, as did C:N mass ratio and lipid mass. Maximum carbon mass within a stage was achieved by copepods at chlorophyll *a* concentrations of around 3 mg m^{-3} (0 - 60 m). These concentrations of phytoplankton were associated with warmer

water, therefore, temperature in the upper water column may have influenced carbon mass indirectly.

In Chapter 6 the carbon mass of individual species stages measured in this study will be used in conjunction with stage duration data from Chapter 4, to estimate the mass specific growth rates. The carbon masses derived here can be used confidently to reflect growth rates *in situ*, as there was no significant loss carbon mass in individuals between their capture and the end of the 48 hour incubations.

Chapter 6 Growth Rates of *Rhincalanus gigas* and *Calanoides acutus*

6.1 Introduction

In order to appreciate the role of copepods in the energy flow in marine ecosystems, it is essential to determine the rates of growth and production and the factors regulating them. Growth may be measured in terms of the proportion of body length increased by per day, or more meaningfully by expressing it as a proportion of the body mass an individual increases by per day. Globally, mass specific growth in marine copepods range from those estimated for the Antarctic species *Rhincalanus gigas* which are as low as 0.0006 d^{-1} (Conover & Huntley 1991) to 0.75 d^{-1} in *Acartia tonsa* (Miller et al. 1977).

In order to calculate growth in copepods an accurate estimate of the increase in body mass over a known period of time is required. Furthermore, in order to gain a mechanistic understanding of how growth is controlled, data on concurrently measured environmental variables, such as food and temperature are needed. This procedure is very time consuming and consequently the majority of the estimates of growth rate in the literature are based on models which draw on the limited field data available (for example see Hirst & Sheader 1997 and references therein). These models are intended to be globally applicable and have been developed so that more easily and readily measured parameters, such as temperature and/or body mass can be used to predict the growth rate of copepods in a particular system (Ikeda & Motoda 1975, Huntley & Lopez 1992, Hirst & Sheader 1997, Hirst & Lampitt 1998). However these models cannot capture the

different physiologies and growth rates of the component species in any given community. So whilst these models are useful in gaining a general figure for copepod growth at different temperatures, species and stage specific measurements are required to understand the role of a particular species in its environment.

More frequently female egg production has been used as a measure of the juvenile mass specific growth rates. Adult females do not grow appreciably in size, and egg production has often been used to represent their growth rates (Hay 1995, McLaren & Leonard 1995, Poulet et al. 1995). The variability in egg production rate of planktonic copepods is an important demographic parameter in any quantitative description of population growth (Ohman et al. 1996). Measuring female egg production in field populations is relatively simple, although the relationship between adult and juvenile production have often not been validated, and more recently Hirst & McKinnon (2001) have suggested that egg production alone may not accurately reflect female growth.

This study presents the first *in situ* measurements of mass specific somatic growth of *Rhincalanus gigas* and *Calanoides acutus* in relation to concurrently measured environmental variables. The station-based data on stage duration and carbon mass reported in Chapters 4 and 5, for specific stages of *Rhincalanus gigas* and *Calanoides acutus*, are used to determine stage and mass specific growth rates. These are then compared to prevailing environmental conditions and finally comparisons between adult and juvenile mass specific growth rates are made.

6.2 Material and methods

Egg production experiments

At each station, where abundance permitted, three groups of 10 adult female *Rhincalanus gigas* and *Calanoides acutus* were sorted from the samples. These were transferred into tubular perspex cylinders closed off at their lower end with 800 µm netting. These were then suspended in 1.5 l glass jars filled with 0.2 µm filtered sea-water and placed in a constant temperature room running at ambient sea-water temperature, and incubated in darkness for 24 hours. At the end of the incubation period eggs were removed and counted and females used for dry and carbon mass analysis (see Chapter 5). Mass specific growth rates of females (g_f) were a measure of the amount of carbon expended in egg production per day, and were calculated using the equation:

$$g_f = W_e / W_a$$

where W_e is the carbon mass of eggs produced over 24 hrs and W_a is adult female carbon mass. Carbon mass and daily egg production rates of the females were station specific; egg carbon mass was estimated using the dry mass reported in Ward & Shreeve (1995) assuming carbon content was 45% of dry mass.

Juvenile growth

Juvenile mass specific daily growth rates (g_j) was assumed to be exponential and were calculated using the data on stage duration and carbon mass (Chapters 4 and 5), using the equation:

$$g_j = (\ln Wi_{+1} - \ln Wi)/t$$

where Wi is the mean carbon mass of stage i (µg C ind^{-1}), Wi_{+1} the mean carbon mass of the subsequent stage (µg C ind^{-1}) and t is the estimated overall stage duration (d).

Stage duration estimates for stage CV *R. gigas* and *C. acutus* were not possible during the current study as this stage was not moulting (see Chapter 4). As they were also not moulting during a spring study in the same area (Ward & Shreeve 1999), it was assumed that individuals remain in stage CV for an extended period of time. Therefore for the purposes of estimating a daily growth rate for stage CV, it was assumed that individuals had remained in stage from late spring (November) to the time of sampling (mid January), a period of about 75 days. This stage duration was therefore used to estimate the daily growth rate of stage CV of both species.

Simple least squares curvilinear and linear regression were used to examine the relationships between female and juvenile g and concurrently measured variables described in Chapter 3.

6.3 Results

Egg production

For *Rhincalanus gigas* and *Calanoides acutus* egg production rates ranged from 0 - 35 and 0 - 40 eggs fem^{-1} d^{-1} with a mean of 11 and 10 eggs fem^{-1} d^{-1} respectively. Egg production rates are presented in relation to chlorophyll *a* and body mass for *R. gigas* and *C. acutus* (Figs. 6.1 & 6.2 respectively). A curvilinear relationship gave the best fit between chlorophyll *a* and egg production, and although there was considerable scatter, was statistically significant (Figs. 6.1a & b). No significant relationship was found between egg production and female body mass in this study (Figs. 6.2 a & b). Mean female mass specific growth rates (ie mass specific egg production rates) were higher in

R. gigas (0.011) than *C. acutus* (0.005) at this time of year (Table 6.1).

Juvenile growth

Juvenile mass specific growth rates of *Rhincalanus gigas* were lower than equivalent stages of *Calanoides acutus* and are presented in Table 6.1. *R. gigas* had a mean value for stages CI - CIII of 0.05 whilst *C. acutus* stages CII - CIV had a mean value of 0.13. A progressive reduction of g was evident in progressively older stages for both species.

Mass specific growth rates of both species are shown in relation to chlorophyll *a* concentration (Fig. 6.3). A curvilinear regression line was fitted as it was anticipated that above a threshold concentration chlorophyll would cease to be limiting. A significant relationship was only found for *Calanoides acutus* stage CIV (r^2 adj. = 0.20, p = 0.004).

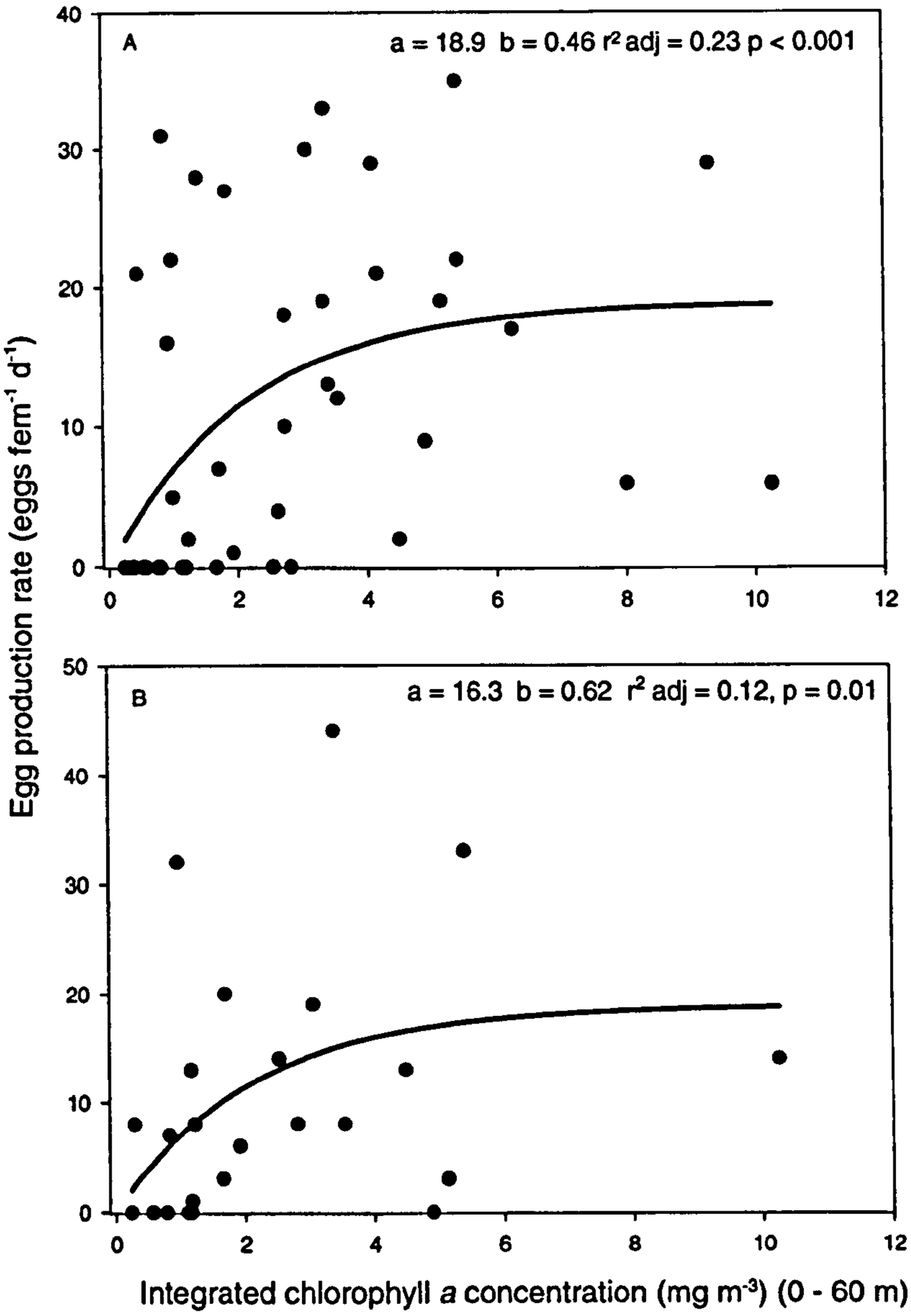
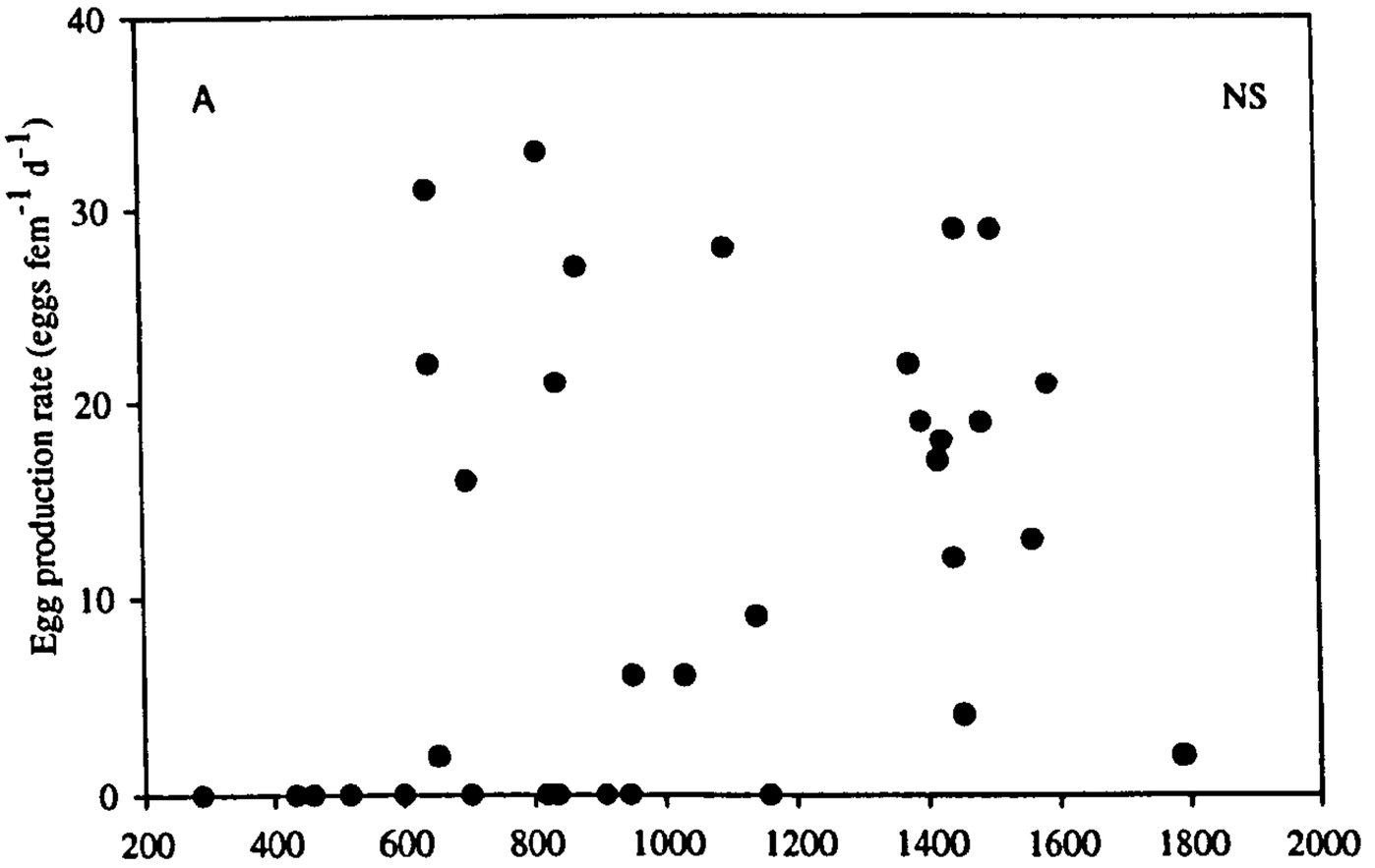


Figure 6.1 A. *Rhincalanus gigas* and B. *Calanoides acutus*. Egg production in relation to Chlorophyll *a* concentration (mg m^{-3} 0 - 60 m). Curvilinear regression line fitted; $EP = a(1-e^{-b*c})$.



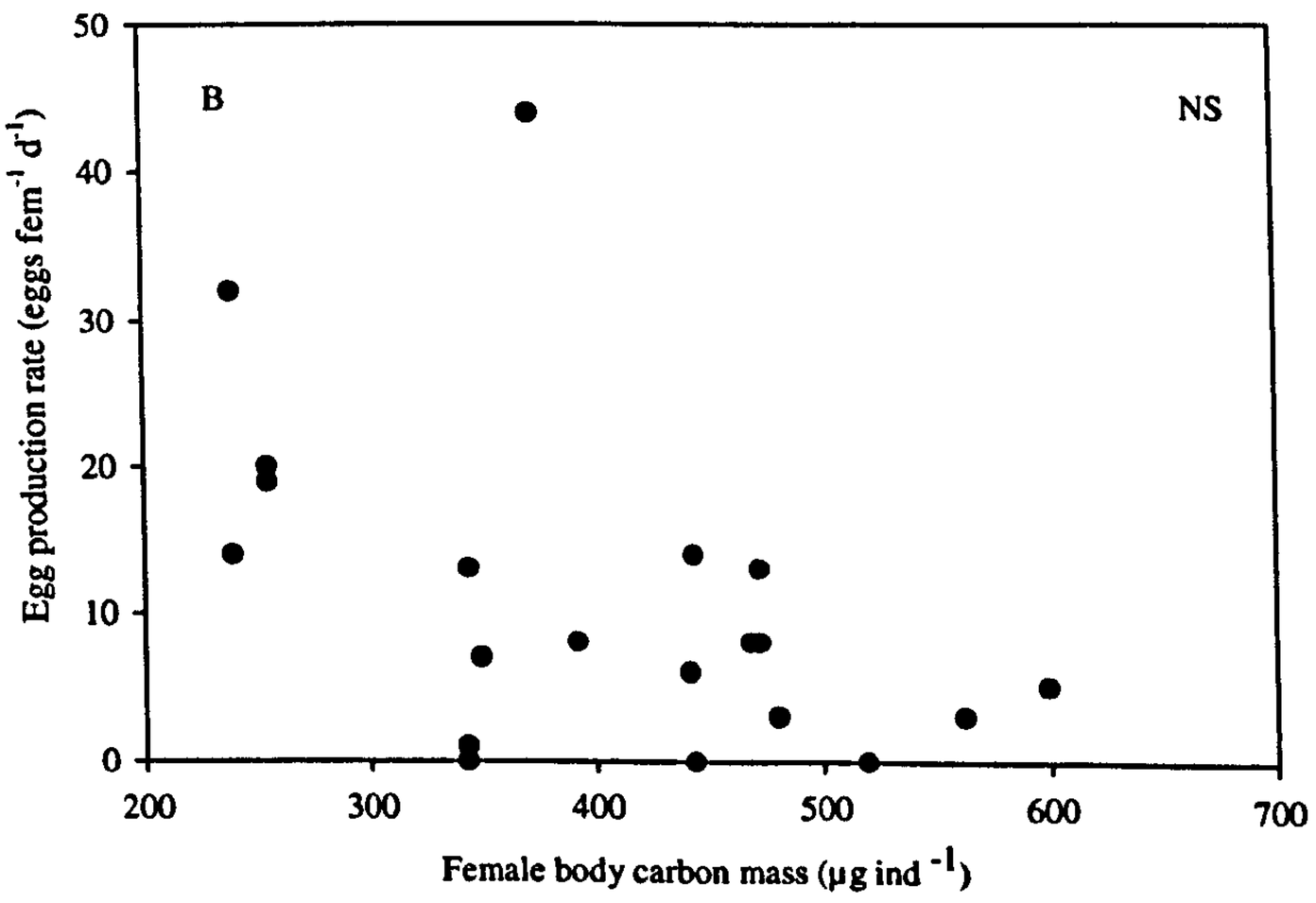


Figure 6.2 A. *Rhincalanus gigas* and B. *Calanoides acutus*. Egg production rate (eggs fem^{-1} d^{-1}) in relation to female body carbon mass. No significant relationship (NS)

Table 6.1 *Rhincalanus gigas* and *Calanoides acutus*. Mean mass specific growth rate (g) for all stations and years. Female production estimated as the amount of carbon expended in egg production per day. Range $_i$, range of growth rates derived from ingestion rates (see p 139 for details).

Copepodite stage	g	Range $_i$
Rhincalanus gigas		
CI	0.060	0.033 - 0.190
CII	0.060	0.018 - 0.150
CIII	0.040	0.013 - 0.110
CV	0.008	0.008 - 0.069
CVI f	0.011	0 - 0.028
Calanoides acutus		
CII	0.240	0.05 - 0.39
CIII	0.150	0.03 - 0.27
CIV	0.140	0.023 - 0.18
CV	0.012	0.024 - 0.19
CVI f	0.005	0.0083 - 0.06

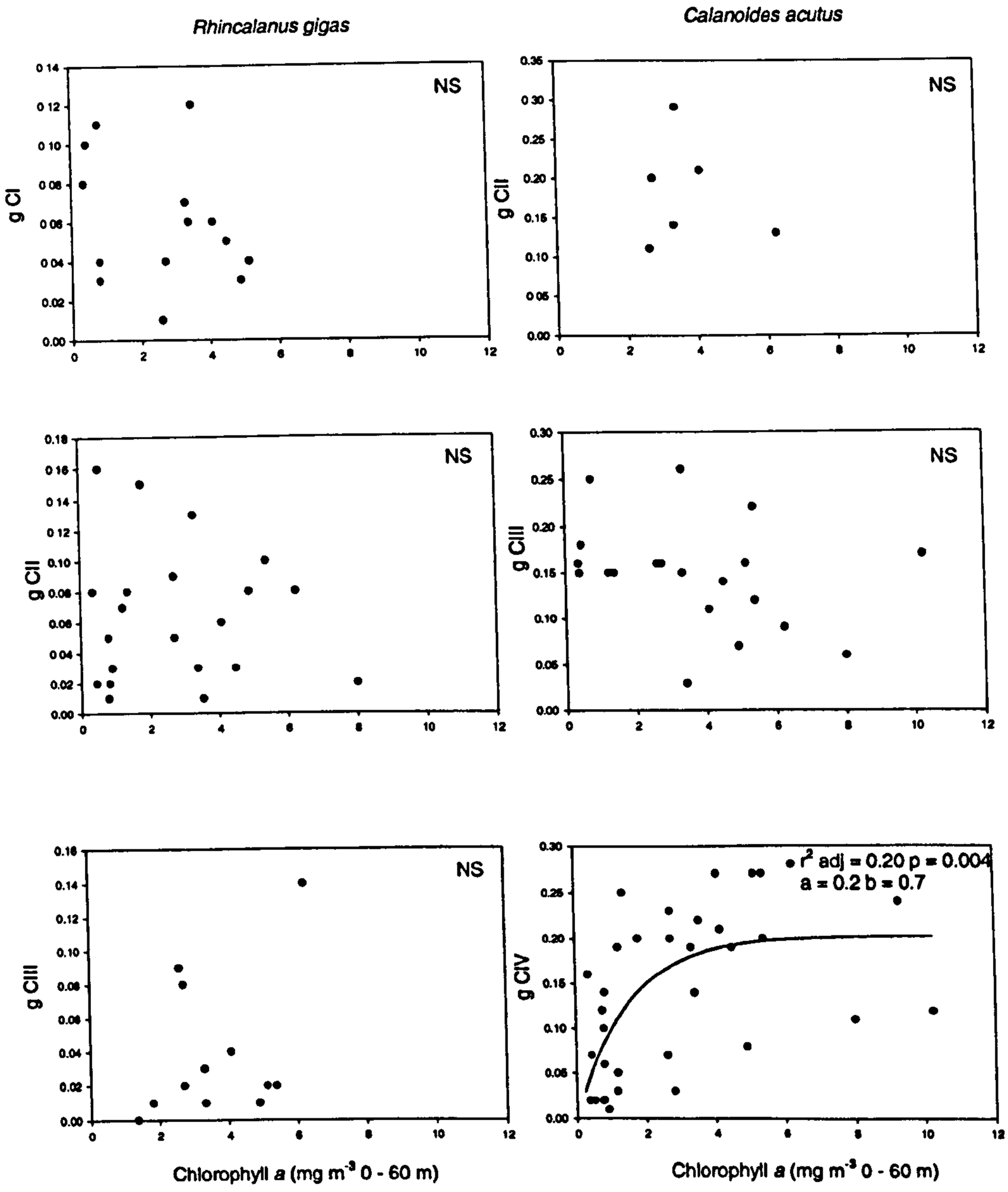


Figure 6.3 *Rhincalanus gigas* and *Calanoides acutus*. Mass specific growth rate (g) in relation to standing stock of chlorophyll *a* (mg m^{-3} 0 - 60 m). Curvilinear regression line fitted $g' = a\,(1-e^{-b*x})$. Not significant NS.

The relationship between silicate concentration and the mass specific growth rates of both species are shown in Fig. 6.4. Both least squares linear and curvilinear lines were fitted; where these were significant they gave similar r^2 and p values, therefore only the linear regressions are shown as fewer assumptions are made about the data in their fitting. A significant negative relationship was found for *Rhincalanus gigas* stage CIII and *Calanoides acutus* stage CIV. Mass specific growth rates of both species are shown in relation to mean temperature in the top 60 m (Fig. 6.5). A significant positive relationship was only found for *C. acutus* stage CIV (r^2 adj. = 0.27, p = 0.0015). Finally the relationship between g and body carbon mass ($\mu g\ ind^{-1}$) was made (Fig. 6.6), a significant relationship was again only found in the case of *C. acutus* stage CIV.

Mean body carbon mass ($\mu g\ ind^{-1}$) for all individuals of a particular species and stage were compared to their mean g presented in Table 6.1 (Fig. 6.7). Stages of the two species with equivalent body carbon mass have differing g, with the younger stages of *C. acutus* having consistently higher g values.

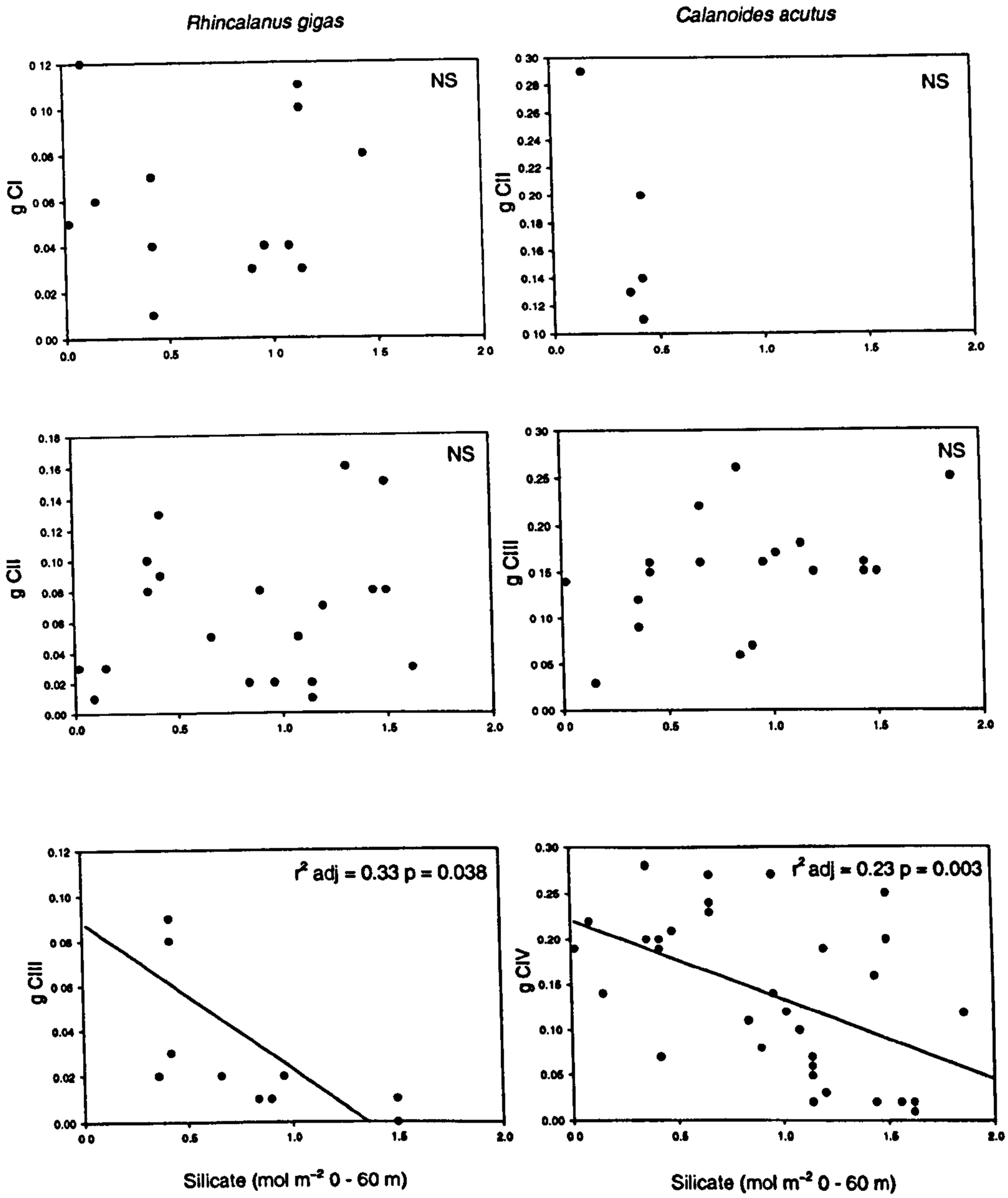


Figure 6.4 *Rhincalanus gigas* and *Calanoides acutus*. Mass specific growth (g) in relation to silicate concentration (mol m^{-2}, 0 - 60 m). Least squares linear regression lines fitted. Not significant NS.

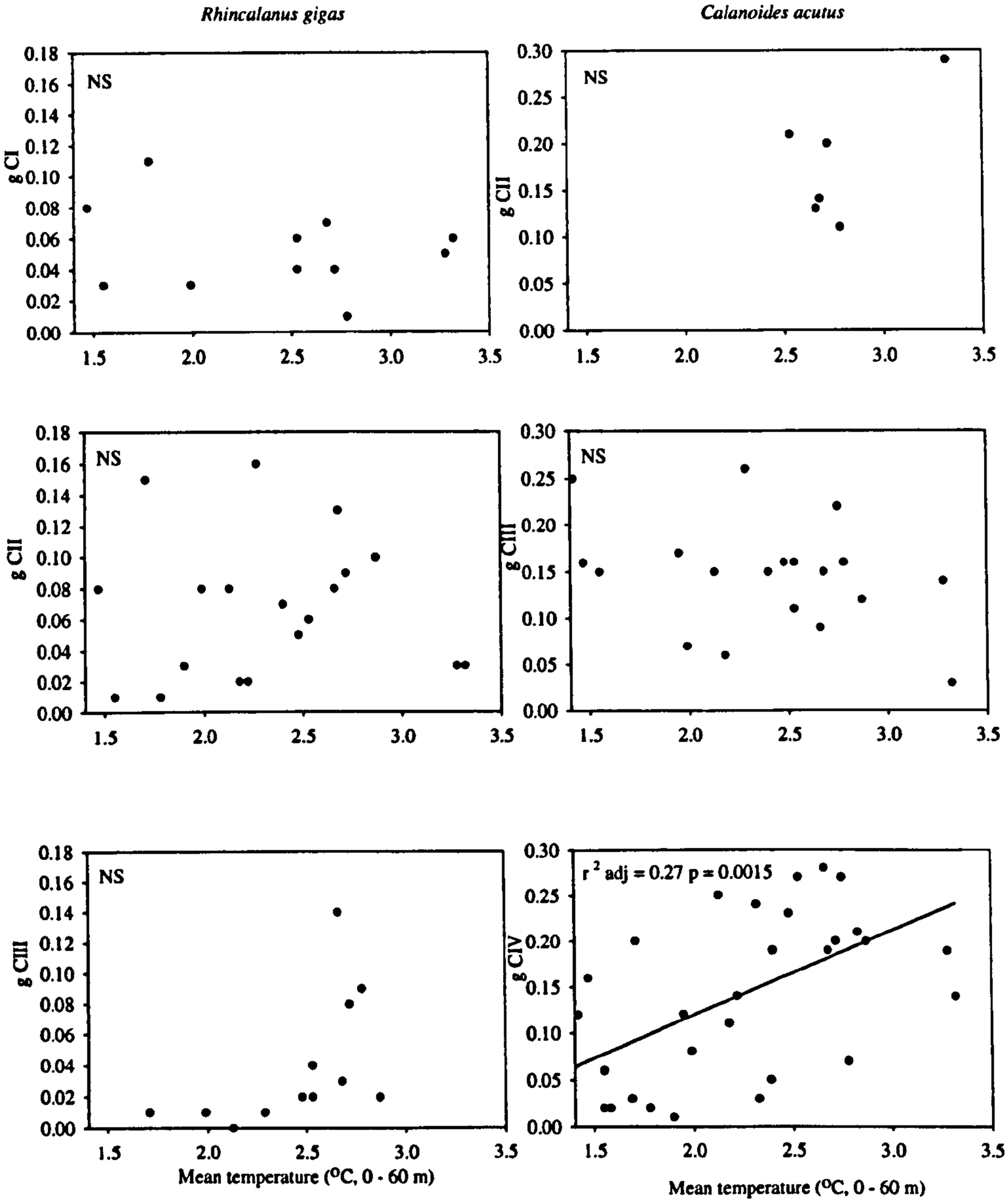


Figure 6.5 *Rhincalanus gigas* and *Calanoides acutus*. Copepodite stages CI - CIII presented for *R. gigas* and stages CII - CIV for *C. acutus*. Mass specific growth rates (g) in relation to mean temperature (°C, 0 - 60 m). Linear least squares regression line fitted, r^2 adj. value presented where applicable, NS not significant.

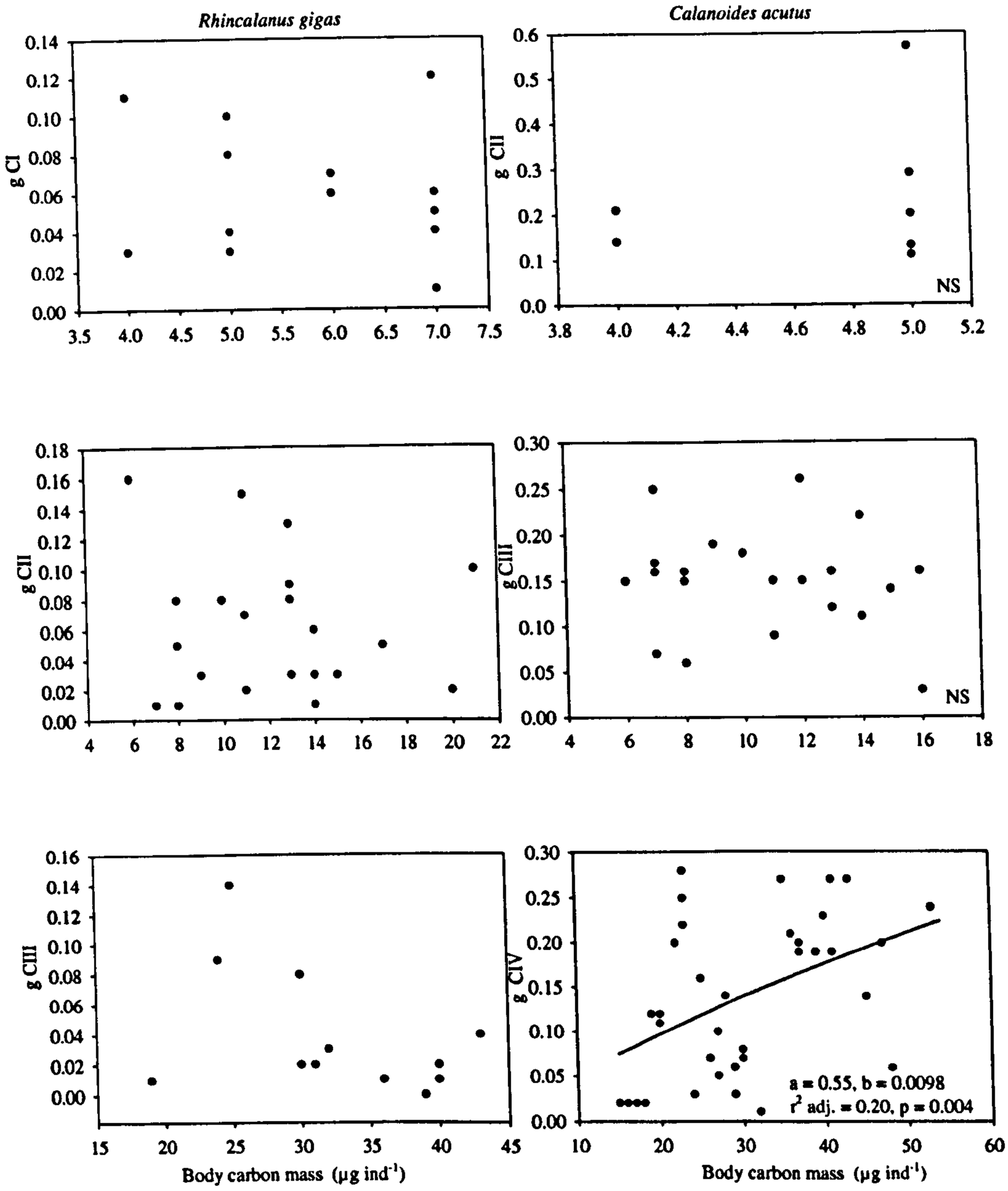


Figure 6.6 *Rhincalanus gigas* and *Calanoides acutus*. Mass specific growth in relation to body carbon mass. Curvilinear least squares regression line fitted. $g = a(1-e^{-b*c})$. Not significant NS.

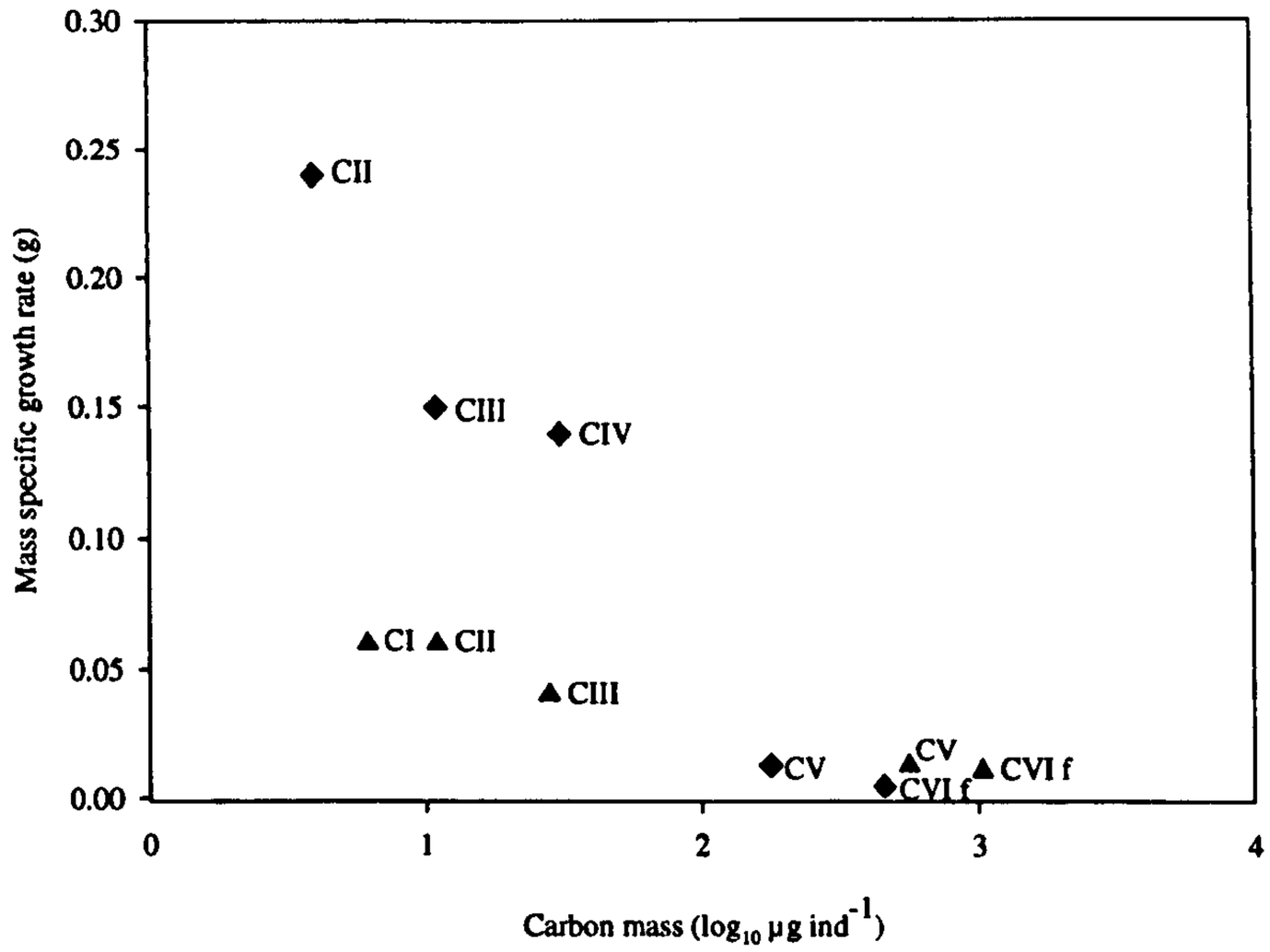


Figure 6.7 *Rhincalanus gigas* (▲) and *Calanoides acutus* (◆). Mean mass specific growth rate for each species in relation to mean carbon mass (log_{10} µg ind $^{-1}$).

6.4 Discussion

The estimates of g for the range of stages of both species are within the range of literature values for copepods growing at similar temperatures (Huntley & Boyd 1984, Huntley & Lopez 1992, Hirst & Sheader 1997, Hirst & Lampitt 1998), although no systematic relationship was found for either species between g and chlorophyll *a*, silicate or temperature. In the present study there was no relationship between stage duration and temperature, which may well be due to the restricted range of field temperatures over which they were measured. However, carbon mass was significantly related to the past feeding environment. Thus, although individuals within a stage may have a similar g, and remain within a stage for a similar duration, the relative daily carbon increment will be significantly more in individuals that have experienced higher food concentration.

Another way to gauge how realistic these estimates are is to compare published ingestion rates with the amount of carbon that would need to be ingested to support these growth rates. Ingestion rates for the younger copepodite stages were derived by scaling those of the older stages (Schnack et al. 1985, Atkinson et al. 1996) using an allometric exponent for mass-specific ingestion rates of 0.377 (Ikeda 1977). It is assumed that the fraction of ingested food available for growth is 50%, as calculated for *Calanus pacificus* from Puget Sound by Vidal (1980a). Although Vidal's experiments were conducted at 8 and 15°C, temperature was shown to have only a slight effect on growth efficiency (Vidal 1980d). The range of growth rates derived in this way for each stage are shown in Table 6.1. In all younger copepodite stages the measured growth rates in this study would be supported adequately by the estimated ingestion rates. The g for adult female *Calanoides acutus* was lower than that predicted from the ingestion rates, however, at the time of sampling *C. acutus* would be past its reproductive peak and may explain these

low growth estimates. However, it has also been suggested by Hirst & McKinnon (2001) that egg production may not accurately reflect an individual females growth.

In the two species of Southern Ocean copepod studied here, mass specific growth rates within a species are body mass dependant. While this appears to be a general rule (cf. Vidal 1980b, Hirst & Sheader 1997, Richardson & Verheye 1998) until now such data have been lacking for polar regions. Importantly this study also highlights clear differences between the two species. The early stages of *Calanoides acutus* have growth rates that are some 2-3 times higher than *Rhincalanus gigas* which is consistent with what we know about the metabolic demands of these two species. Thus the mass specific respiration rates (Schnack et al. 1985) and ingestion rates (Atkinson et al. 1992) of the older stages of *R. gigas* are reported to be lower than for *C. acutus*. Mass specific growth decreased with progressively older stages for both species studied. This pattern is in agreement with growth data for *Calanus agulhensis* on the Agulhas bank (Peterson & Hutchings 1995) and *C. pacificus* in controlled laboratory experiments (Vidal 1980a).

Comparison of juvenile growth and female egg production rate

In previous studies, egg production has often been used as a measure of juvenile growth (Hay 1995, McLaren & Leonard 1995, Poulet et al. 1995), although McLaren & Leonard (1995) do caution the general use of this relationship without further verification. Peterson et al. (1991) criticised the use of female egg production rates as a measure for juvenile growth, suggesting that in their study on *Calanus finmarchicus* in the Skagarrak this assumption would have underestimated juvenile production by a factor of 2.7. The current study supports this caution as the mass specific growth rates of females were much lower than those measured in the younger copepodite stages, whose

g was variable with stage. Egg production (EP) showed a curvilinear relationship with chlorophyll *a* concentrations, suggesting food limiting conditions at the lower concentrations, but that above ~3 mg chl *a* m^{-3} (0 - 60 m) reached maximum levels for this period of the year. Younger copepodite growth however appeared unaffected by food concentration. Thus the timing of the EP studies in relation to the peak of female reproductive effort will affect estimates of growth.

In the next chapter the population structure and abundance of *Rhincalanus gigas* and *Calanoides acutus,* as well as the total copepod abundance are studied in relation to environmental factors. Mortality rates are also estimated for a range of the copepodite stages of the two key species. Estimates are made using the model constructed by Asknes & Ohman (1996) which uses data on the abundance of individuals in two consecutive stages, combined with an estimate of their stage durations.

Chapter 7 Copepod population dynamics

7.1 Introduction

Demographic processes in Southern Ocean copepod populations are only too frequently interpreted from 'snapshots' of the system. Logistical constraints of sampling in polar seas generally preclude the collection of time-series data, which in other areas may provide accurate information on events such as the initiation of the spring bloom, timing of copepod reproduction and duration of productivity within the system. A single 'snapshot' integrates events that have occurred in the days, weeks or months preceding the period of sampling, making interpretation more difficult. However, instantaneous data (copepod species composition, their abundance and population stage frequency) may be combined with independent information on stage durations and egg production rates in relation to concurrently measured environmental data, to provide a temporal context for ecological processes. This approach has been used to link the abundance and stage frequency data of the two study species to environmental parameters. Stage durations and egg production rates, presented in Chapters 4 and 6, are used to estimate both the magnitude and duration of recruitment in relation to estimates of the timing and duration of the bloom. These were then related to the whole copepod population.

Whilst reproduction and growth are two important aspects of copepod population dynamics and have been the focus of many studies, mortality has received much less attention. Mortality has a profound influence on population structure and it is hard to assess in populations where recruitment and/or advection are occurring. It has been

suggested that mortality, rather than fecundity, may operate to control the size of a population, as demonstrated for *Pseudocalanus newmani* by Ohman & Wood (1995, 1996) and Ohman et al. (1996). This contrasts with a study by Mullin (1991) who found that rates of egg production rather than mortality control population numbers of *Calanus pacificus* and *Rhincalanus nasutus*. However, the mode of egg laying may influence this, as *P. newmani* brood their eggs in sacs until the nauplii develop whilst *C. pacificus* and *R. nasutus* broadcast their eggs directly into the water. Rates of predation on both the eggs and nauplii stages may be high, so brooding may significantly reduce the mortality rate during this early stage.

Some studies have found that mortality is lower in younger developmental stages, and it is suggested that this is because they remain within a stage for a shorter period of time (Castel & Feurtet 1992). Conversely Gaudy (1992) found lower rates of mortality in the older stages of *Acartia tonsa* in a Mediterranean lagoon, and suggested that this was because there was a lack of significant predation in these stages. Rates of mortality have been derived for a number of species, with literature values ranging from 0.535 d^{-1}, derived for copepodite stages of *Eurytemora affinis* in the Gironde Estuary (Castel & Feurtet 1992), to 0.003 d^{-1} for stages CIII - CVI *Calanus hyperboreus* in Karsfjorden, western Norway (Matthews et al. 1978).

Rates of mortality can be estimated from stage frequency data, using one of two methods. Most commonly estimates are made from time series of abundances, which has been coined as the 'horizontal life table approach' by Aksnes & Ohman (1996). This method requires many samples to be taken over a period of weeks to months, from an area in which advective processes have a negligible effect. This method does not lend itself to the Southern Ocean, because the remoteness of the area causes logistical

constraints which generally only allow 'snapshots', of roughly one month duration, of a system generally displaying high advection rates. A second method, coined the 'vertical life table approach' (Aksnes & Ohman 1996), requires only one sample from which to gather stage frequency data, and an independent estimate of stage duration. An assumption made when applying this method is that there is continuous reproduction within the population. Protracted spawning has been reported during the summer months for both *Rhincalanus gigas* and *Calanoides acutus* (Marin 1988), and this method was therefore deemed suitable to apply to this data set in order to give the first estimates of mortality of the younger copepodite stages of these two species

7.2 Materials and methods

Copepod abundance

Zooplankton was sampled using a Bongo net as described in Chapter 3. The preserved samples from the 200 µm mesh nets were subsampled with a Folsom plankton splitter and analysed under a dissection microscope. All copepod taxa were enumerated and copepodite stage frequency determined for the biomass dominant copepods (*Rhincalanus gigas*, *Calanoides acutus*, *Calanus simillimus* and *Calanus propinquus*). Mean age of the *Rhincalanus gigas* and *Calanoides acutus* populations (S) were calculated according to the equation:

$$S = \frac{n\mathrm{CI} + 2n\mathrm{CII} + \ldots + 6n\mathrm{CVI}}{\mathrm{N}}$$

where nCI, $2n$CII, ..., are the number of respective stages and N the total number of individuals of all stages.

Zooplankton community composition data were analysed using the 'Plymouth Routines in Multivariate Ecological Research' (PRIMER ®) package. A species by station similarity matrix was constructed from the station-based copepod abundance data (ind m^{-2}, 0 - 200 m), using three data sets. The first set (A) included all species and stages of copepod to which either a square root or a $\log_{10}$ transformation was applied to reduce the over emphasis of the most abundant taxa, thus focussing on any difference in species composition. The second set (B) contained just the copepodite stages of *Rhincalanus gigas* and *Calanoides acutus* with no transformations applied and set C contained all copepod species minus all stages of *R. gigas* and *C. acutus*, with no transformation. Set A was used to see if there were any significant differences in species composition of copepods over the study period. Set B was used to look at differences in the abundance of the copepodite stages of *R. gigas* and *C. acutus*. Set C was used to compare the rest of the copepod assemblage abundance with the two main species used in this study.

The data from each set were then clustered and non-metric multi-dimensional scaling (MDS) applied. The significance of the resultant groupings was tested using the non-parametric permutation procedure ANOSIM (analysis of similarity). In addition, year and station location (on- / off-shelf, western or eastern mesoscale box), were tested for their effect on the separation of stations. ANOSIM determines the test statistic 'Global R', which generally lies within the range from 0 to +1. Where R = 1, all replicates within groups are more similar to each other than to any replicates from other groups. Conversely where R = 0 similarities between and within groups will be approximately the same. On this basis the most appropriate groupings were identified and the SIMPER program (similarity percentages), applied to disclose the species or stages most responsible for similarity within, as well as dissimilarity between groups.

Data sets B & C then had the BIOENV (matching biotic to environmental patterns) procedure applied to test which environmental factors best described the station groupings. Six factors were used as described previously in Chapter 3: Julian date, minimum temperature (°C) in the top 200 m, mean temperature (°C) in the top 60 m, $\log_{10+1}$ transformed krill biomass (g m^{-2}, 0 - 250 m), silicate concentration integrated over the top 60m (mol m^{-2}), and chlorophyll *a* concentration integrated over the top 60m (mg m^{-3}).

Mortality

Mortality estimates were made for *Rhincalanus gigas* stages CI - CIII and *Calanoides acutus* stages CII - CIV using the abundance data for each stage at each station and the mean duration for each stage presented in Chapter 4. Mortality was solved for two juvenile stages by applying equation 7a from Aksnes & Ohman (1996):

$$[\exp(ma_i)-1] / [1-\exp(-ma_{i+1})] = ri$$

where m is mortality of stage $_i$, and a is the stage duration of stage $_i$, ri is the ratio between abundances of two consecutive stages (i and $i+1$).

7.3 Results

Species composition in total copepod assemblage 'Set A'

Total copepod abundance varied approximately 30 fold between stations over the four cruises (14, 000 to 337,000 individuals m^{-2}, 0 - 200 m, (as shown in Chapter 3, Fig. 3.19)). The increase in abundance of total copepods was not dominated by one particular group, as the proportionate share of each grouping remained broadly the same. Table 7.1 shows the proportion of the numerically dominant copepods species (*Oithona* spp,

Ctenocalanus spp, *Metridia* spp. *Calanus propinnquus* and *C. simillimus*, and stages CIV - CVI and CI - III of *Rhincalanus gigas* and *Calanoides acutus*) in relation to the total copepod abundance. These six groups accounted for at least 80 % of the total copepod abundance.

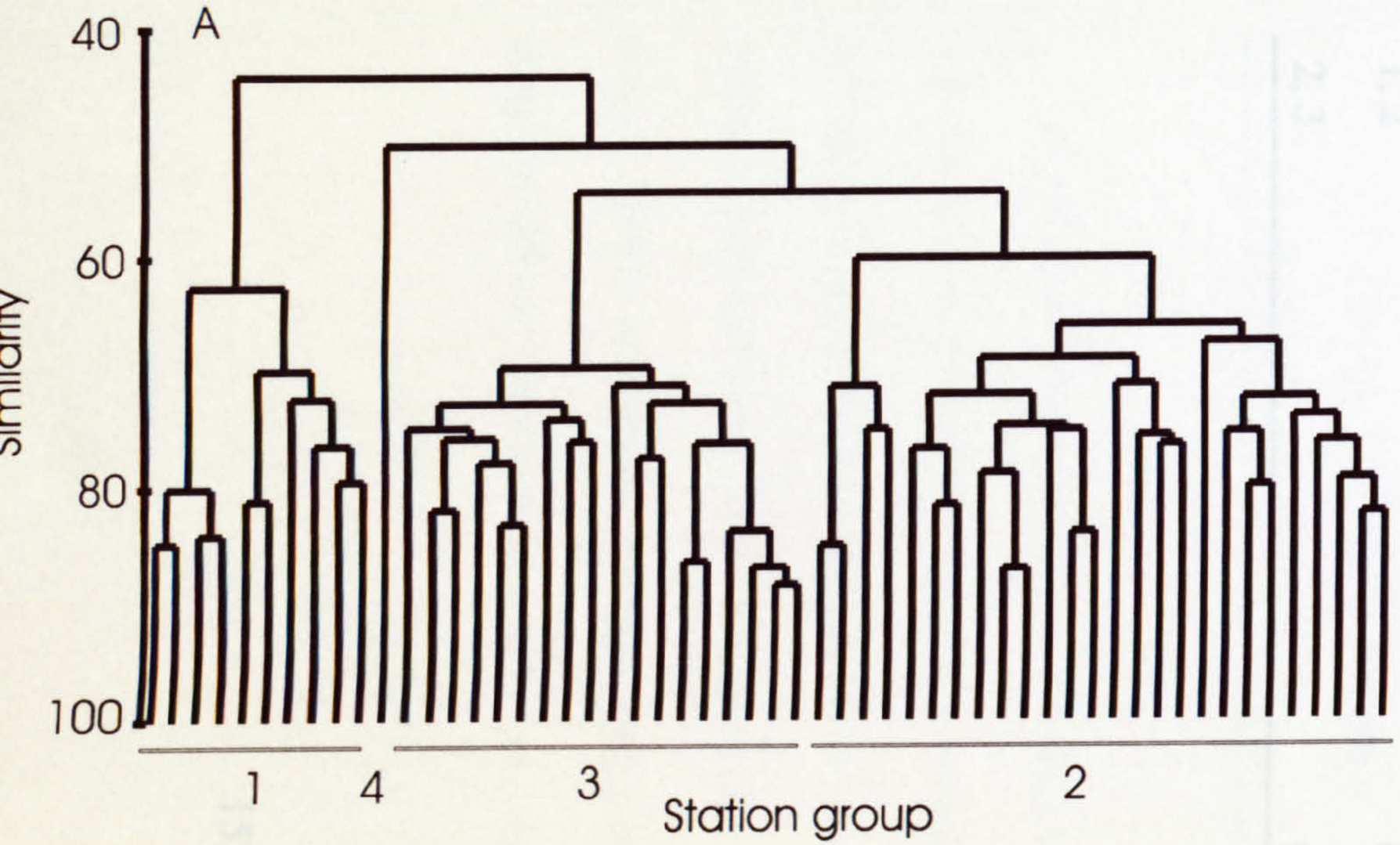
Table 7.1 Copepod species as a proportion of the total copepod abundance (TCA), TCA individuals (thousands) m^{-2} to a depth of 200 m. All copepodite stages of *Oithona* spp. (Oi), *Ctenocalanus* spp. (Ct), *Metridia* spp. (Met), *Calanus propinquus* (Cp)/*C. similiumus* (Cs); *Rhincalanus gigas* (Rg) and *Calanoides acutus* (Ca) grouped into copepodite stages CIV - CVI and CI - III. Cumulative % (%) indicates the proportion that these six groups represent of the total copepod abundance.

	Each species as a proportion of the total copepod abundance (%)						
TCA	Oi	Ct	Met	Cp/Cs	Rg/Ca IV-VI	Rg/Ca I-III	%
14 - 67	71	11	6	1	3	1	93
67 - 120	63	14	11	2	4	1	95
120 - 173	48	22	11	2	2	1	86
173 - 226	44	16	12	4	5	3	84
226 - 279	39	23	14	6	3	3	88
279 - 332	45	18	15	6	2	3	89
332 - 385	35	18	22	3	1	2	81
385 - 440	49	22	18	1	1	1	92

Cluster analysis of data set A across all 4 years revealed 3 main station groupings with a single station remaining unaccounted for by any of the main groupings (Fig. 7.1a). ANOSIM indicated that there were significant ($p < 0.01$) differences between the four

station groups. Tests using year and station location, (on- or off- shelf, east and west) as a basis for grouping the data, or as additional factors in two way crossed and nested analyses, indicated only weak differences with respect to R, and overall the initial separation was most robust (Table 7.2). It is possible for R to be significantly different from zero yet be small as a result of the many replicates within each group, so attention should be focussed more on the value of R than its significance. The data were further tested by SIMPER. This indicated that changes in the abundance of 2 main species, *Oithona* spp and *Ctenocalanus* spp., were principally responsible for the similarities within as well as the dissimilarities between all groups (Table 7.3) (group 4 had only one representative and so was omitted from this summary). MDS produced an ordination with low stress (0.09) indicating that the stations were well represented in 2D ordination space (Fig. 7.1b). Analysis indicated that the zooplankton community was essentially the same across all years and regions, and the principal difference lay in the abundance of the major components, the station groups lay along a gradient with group 1 being characterised by low abundance and group 3 the highest (Fig.7.1c). Log transformation of the original species data matrix to further reduce the importance of abundant taxa followed again by clustering produced essentially the same story; a similar grouping of stations which MDS indicated was still a reasonable representation of the data (stress 0.17). ANOSIM confirmed significant differences between these station groups which were again more robust than partitioning of the data in any other way, and which were again related to changes in abundance. SIMPER indicated that the differences between groups were now driven by different taxa, principally the younger stages of the biomass dominant species *Rhincalanus gigas* nauplii - CII, *Calanus simillimus* CI - III and *Calanoides acutus* CI - III.

Figure 7.1 A Bray-Curtis Similarity Cluster based on total abundance of all copepods (square root transformed) 'station groups 1 - 4 were arbitrarily designated based on a similarity level of < 64 %. B MDS ordination of the station groups identified in the cluster analysis, group 1 (▲), group 2 (■), group 3(▼) and group 4 (◆). C and D, Bubbleplot representations of total copepod abundance (C) and silicate concentration (D). Data have been superimposed on the 'station groups' ordination space (see Fig 7.1B) to clarify spatial relationships. The size of the bubbles are scaled to the minimum and maximum values for each variable, total copepod abundance 14594 - 376141 ind m^{-2}, 0 - 200 m, silicate 0.024 - 2.04 mol m^{-2}, 0 - 60 m.


A
40
60
80
100
Similarity
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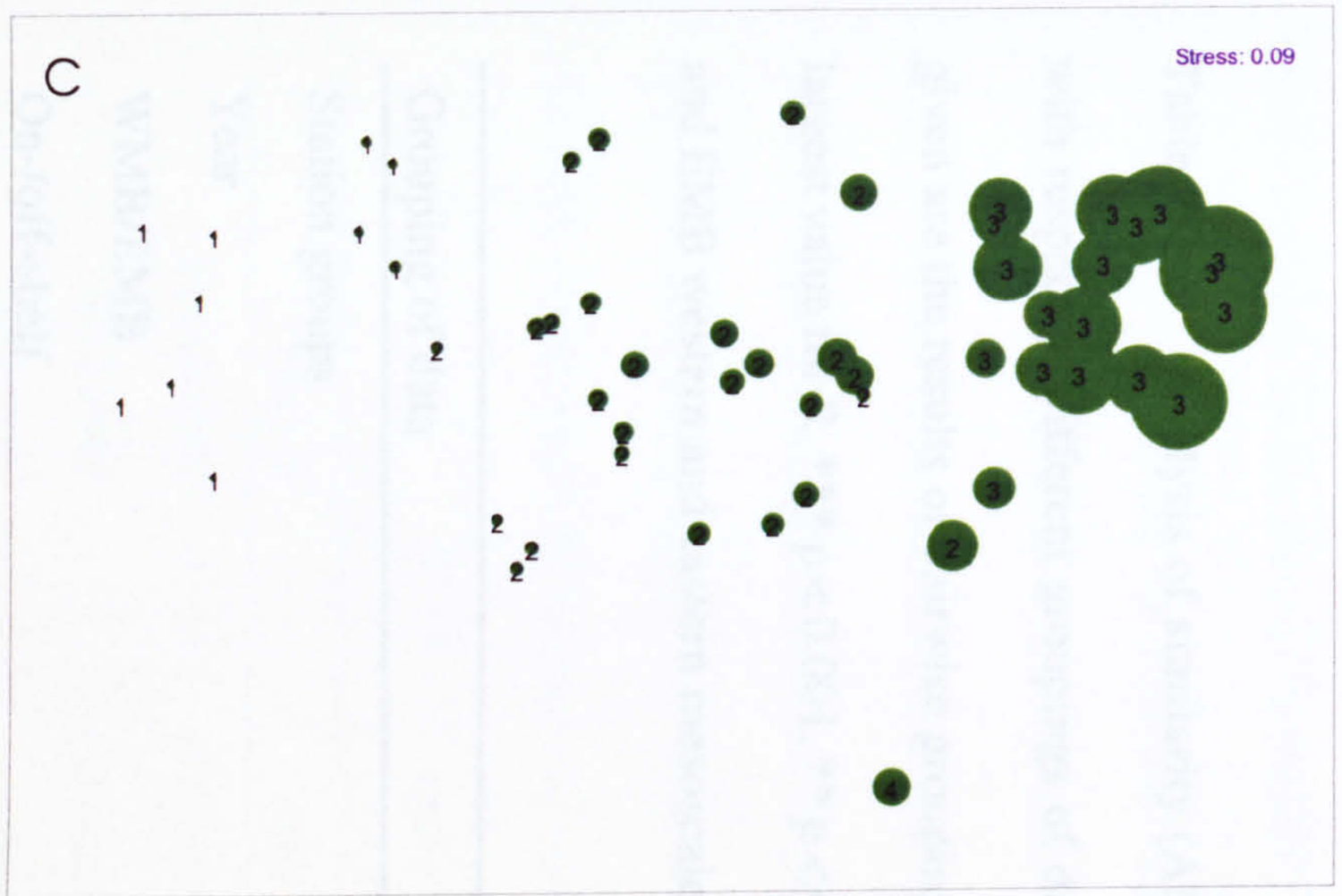
Station group


C
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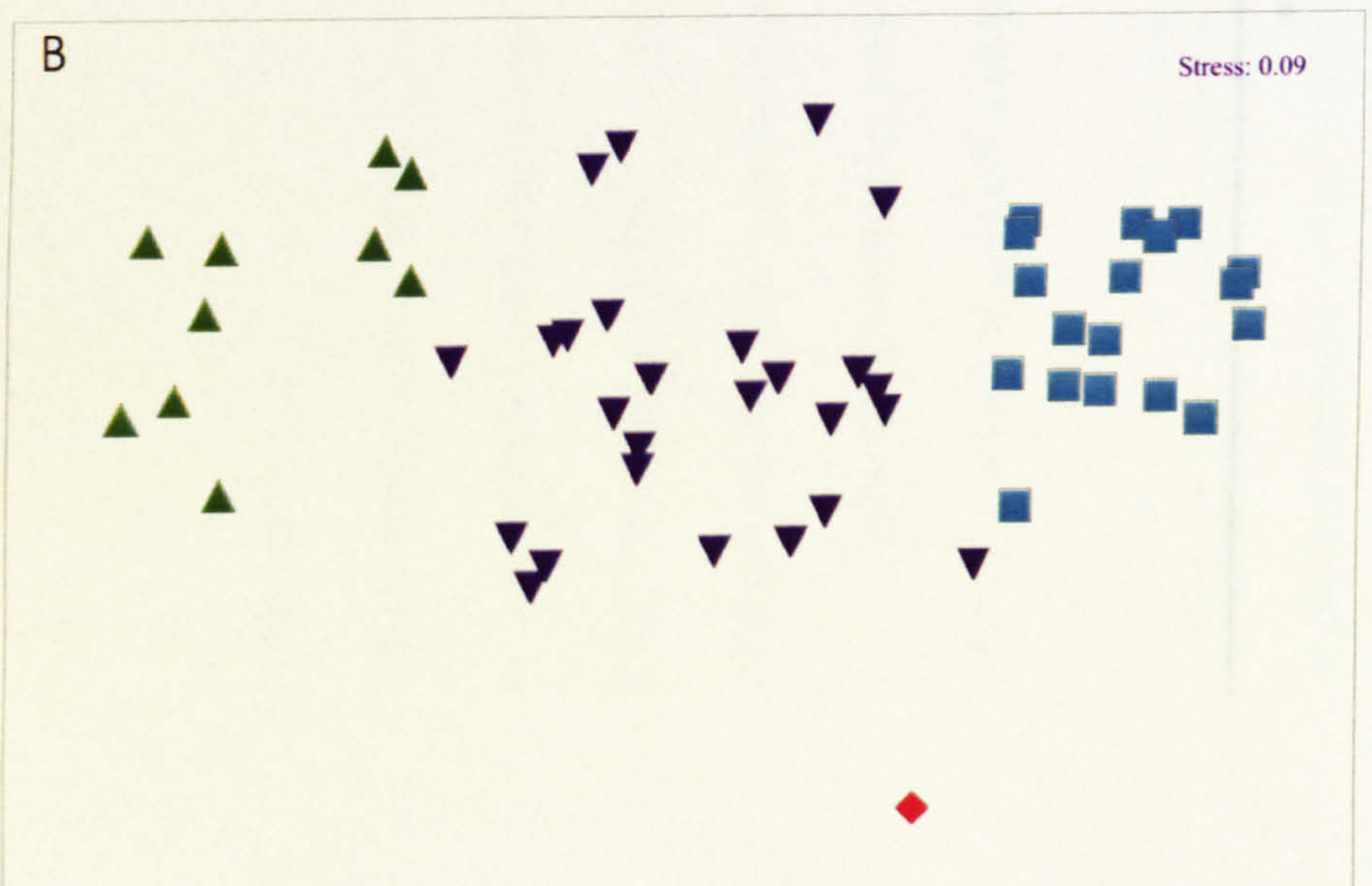
B
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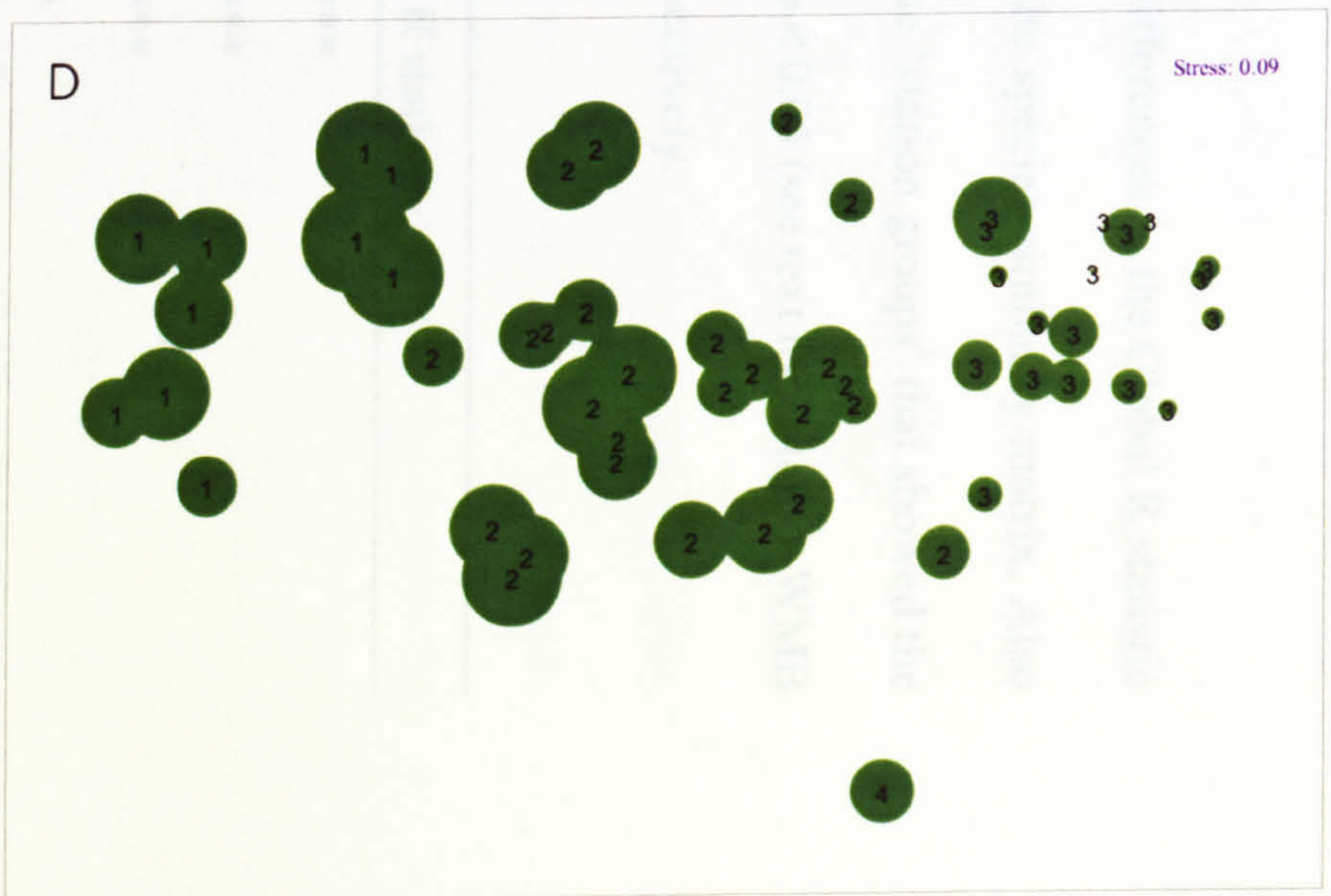
D
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Figure 7.1

Table 7.2 Analysis of similarity (ANOSIM). Differences in the Global R statistic with respect to different groupings of data within the species similarity matrix. Also given are the results of pairwise groupings within the 'station groups' that showed the largest value for R. *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$ (see text for details). WMB and EMB western and eastern mesoscale boxes respectively.

Grouping of data	Global R statistic
Station groups	0.819 ***
Year	0.434 ***
WMB/EMB	0.263 ***
On-/off-shelf	0.095 *
Station groups	R: pairwise tests
1, 3	1.00 ***
1, 2	0.73 ***
2, 3	0.76 ***

Table 7.3 Similarity percentages (SIMPER) carried out on the three main station groups identified in set A (group 4 had only one representative and so was omitted from this summary). The mean abundance (no. ind m^{-2}, 0 - 200 m) within species groups is given for the five species within each group that contribute most to the percentage similarity within that group. ( )[1] = number of samples within each species group. () = rank order within each group. For each group 89 - 94 % of the within group similarity was contributed by the first five ranked species. The proportion of dissimilarity between groups brought about by these species is in the range 77 - 82 %.

'Station group'	1 (10)[1]	2 (26)[1]	3 (18)[1]
Oithona spp.	22285 (1)	53960 (1)	100144 (1)
Ctenocalanus spp.	2113 (2)	12328 (2)	49642 (2)
Metridia spp. CI-III	550 (3)	8138 (3)	32252 (4)
R. gigas CVI f	249 (4)		
Scolecithricella spp.	348 (5)		
C. acutus CV	2748 (4)		
Copepod naupliar	4480 (5)	43990 (3)	
R. gigas naupliar		17434 (5)	

Stage Frequency in Rhincalanus gigas and Calanoides acutus populations (Set B)

Female abundance of both *Rhincalanus gigas* and *Calanoides acutus* was not significantly different between the four years of the survey, (ANOVA for *R. gigas* F = 2.68, p = 0.056; *C. acutus:* F = 2.62, p = 0.061; Fig. 7.2 a&b). However total numbers of *Calanoides acutus* copepodite stages and total copepod abundance overall, varied significantly between years (Figs. 7.3a and 7.4), as to a lesser extent did *Rhincalanus gigas* (Fig 7.3b).

Figure 7.2 Female abundance ± standard deviation (SD) in relation to year. ANOVA A *Rhincalanus gigas* F = 2.68 p = 0.056 and B *Calanoides acutus* F = 2.62 P = 0.061. N = number of stations sampled. Mean (X) with individual 95% confidence intervals (------).

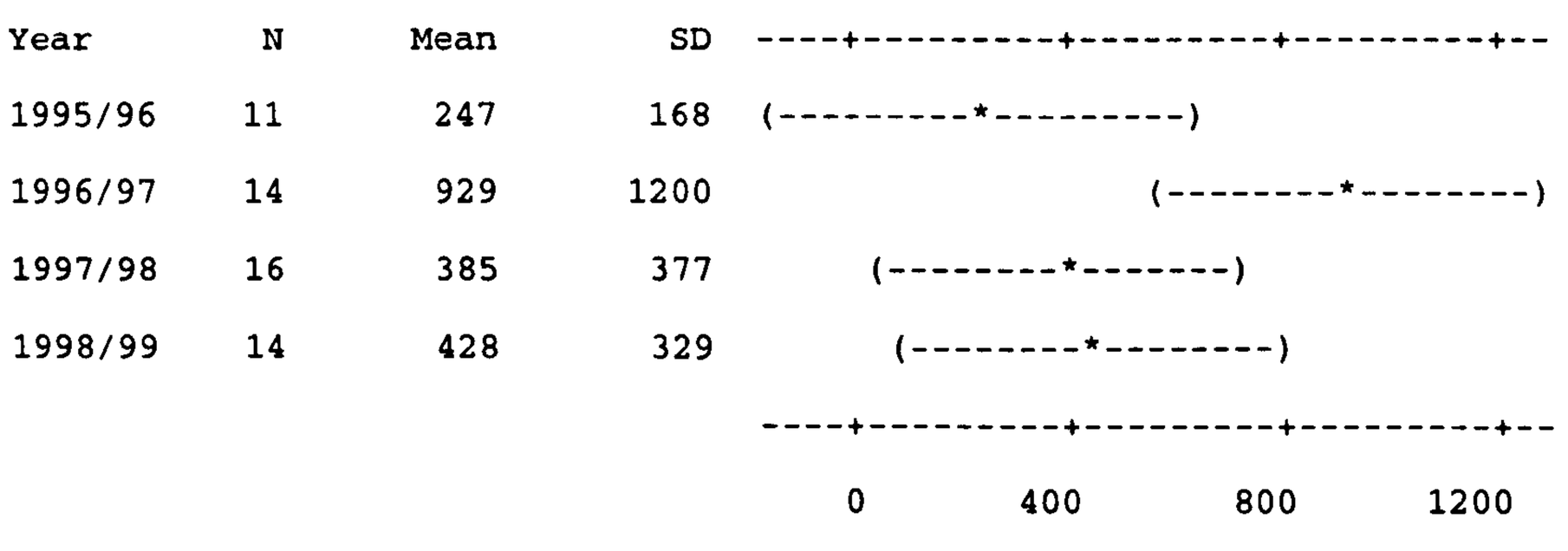


Figure 7.3 Total number of individuals in relation to year. ANOVA A, *Calanoides acutus* F = 4.08, p = 0.011. and B. *Rhincalanus gigas* F = 2.82, p = 0.048. Mean (*), 95% confidence interval (-----).

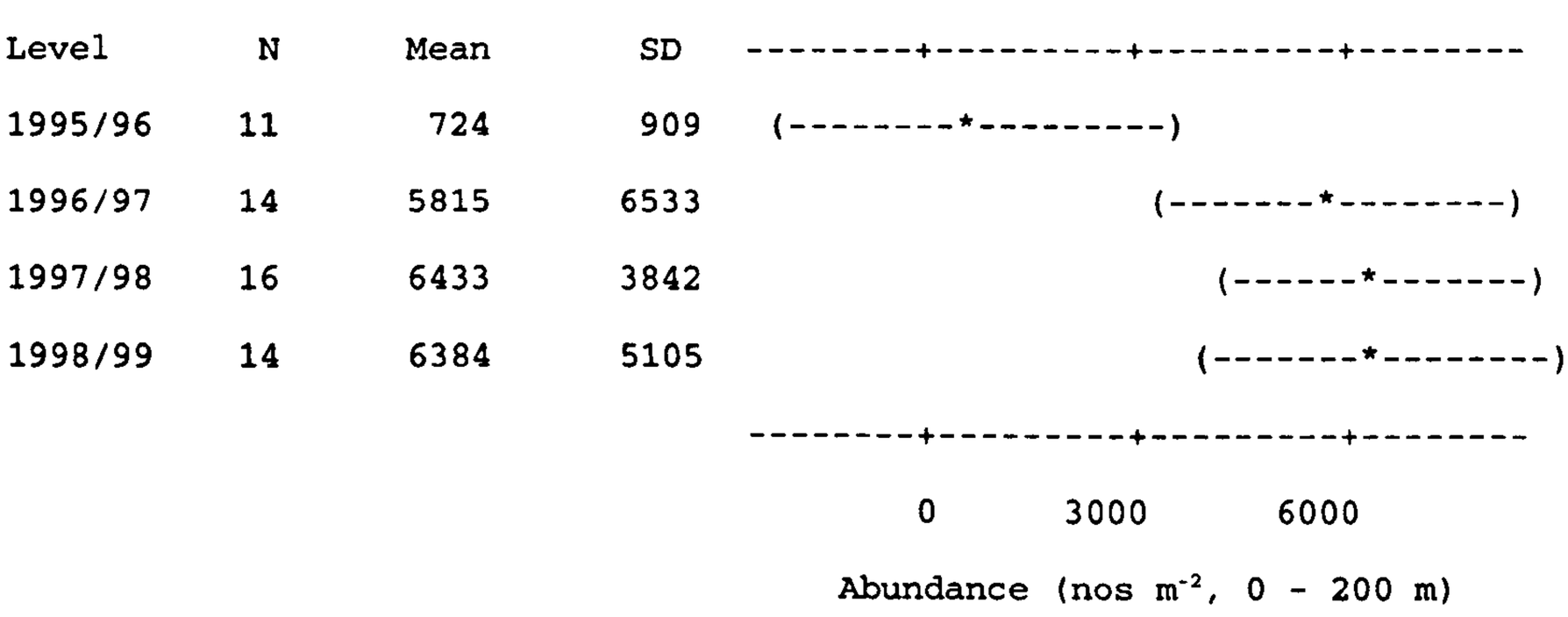
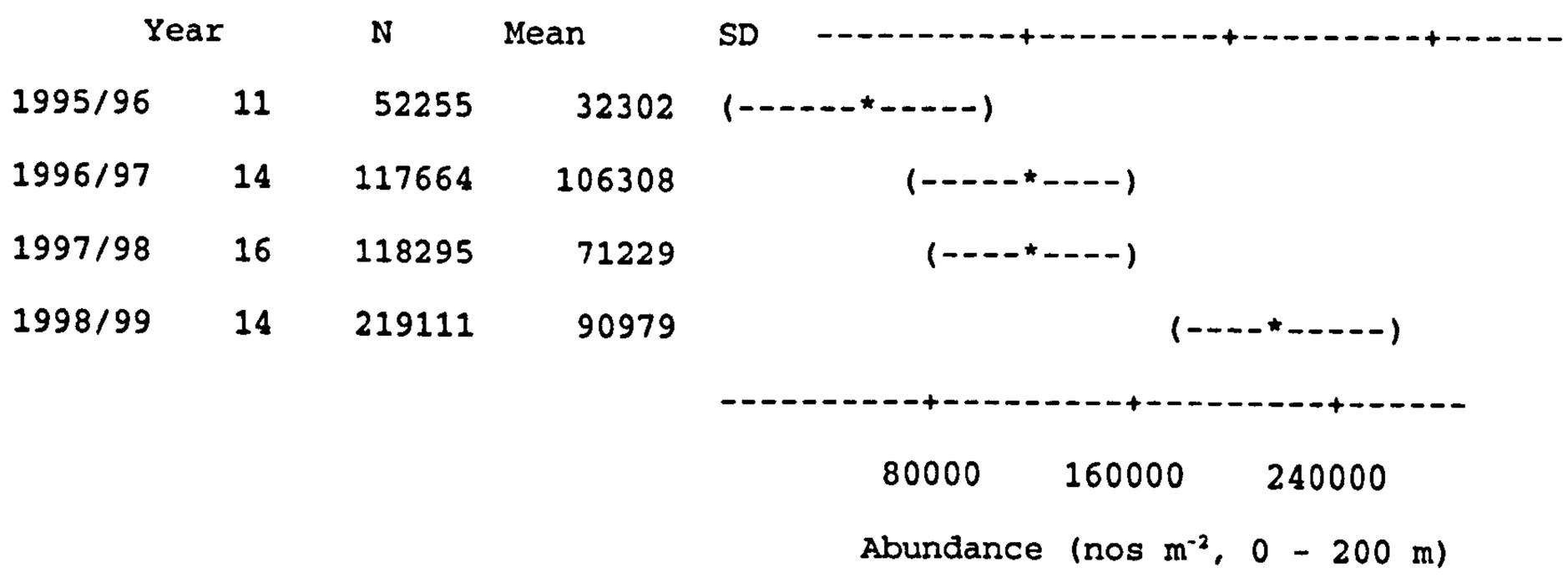


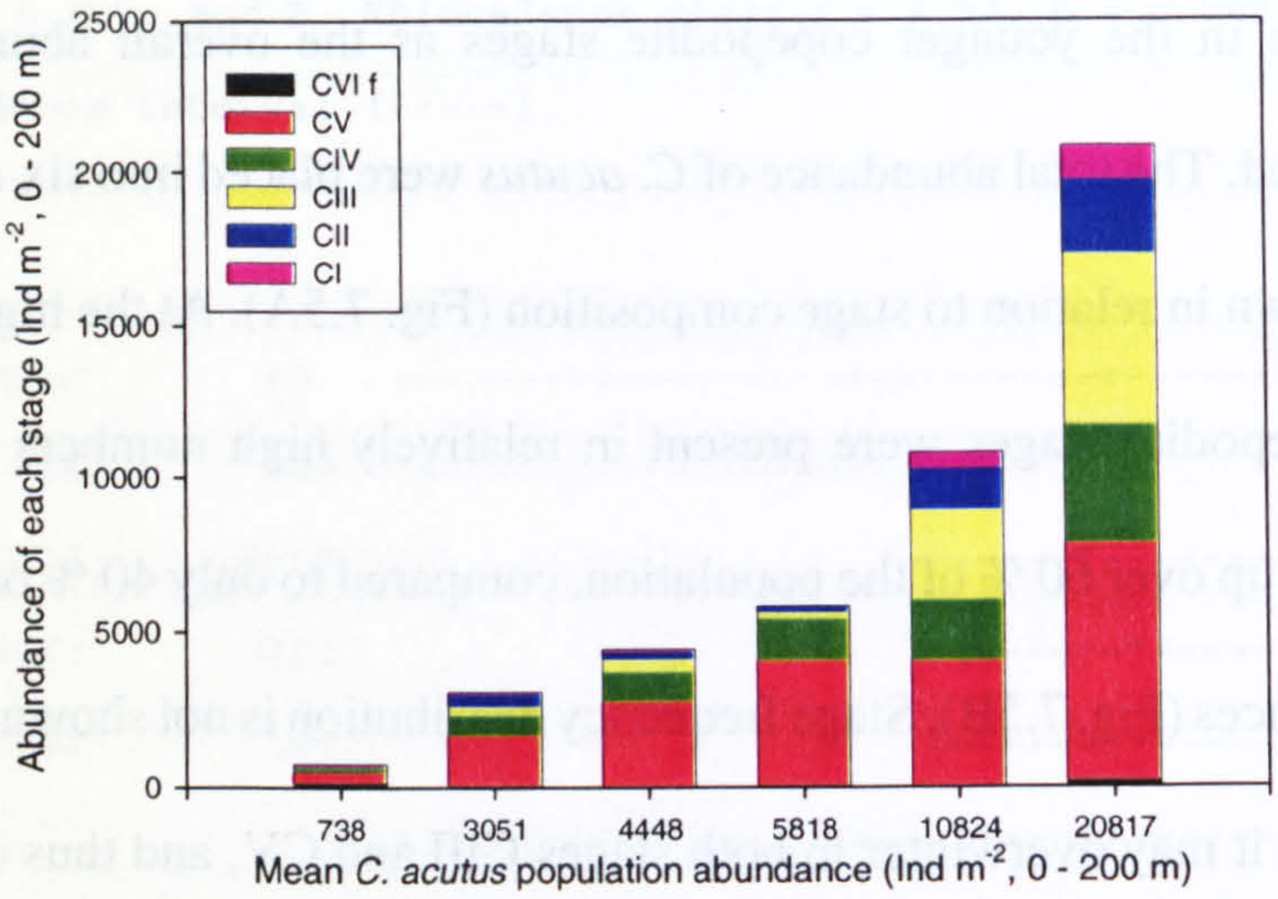
Figure 7.4 Total copepod abundance in relation to year. ANOVA F. = 9.07, p < 0.0001



The population stage frequency of *Calanoides acutus* shows a proportionate increase in the younger copepodite stages as the overall abundance of the species increased. The total abundance of *C. acutus* were placed into six arbitrary groups which are shown in relation to stage composition (Fig. 7.5A). At the highest total abundances, all copepodite stages were present in relatively high numbers with stages CI - CIV making up over 60 % of the population, compared to only 40 % or less at the lower total abundances (Fig. 7.5B). Stage frequency distribution is not shown for *Rhincalanus gigas* because it may overwinter in both stages CIII and CV, and thus confuse the picture of which stages has come from which generation.

Mean age of the population of *Calanoides acutus* was significantly and positively related to silicate concentration (Fig. 7.6). Thus a younger population exists where silicate is reduced due to higher past levels of primary production. *Rhincalanus gigas* did not show such a relationship, most probably because it overwinters as both stage CIII and CV (Ward et al. 1997), which serves to average the mean age of the population over two years and hence cloud the relationship with current silicate concentrations.

A



B

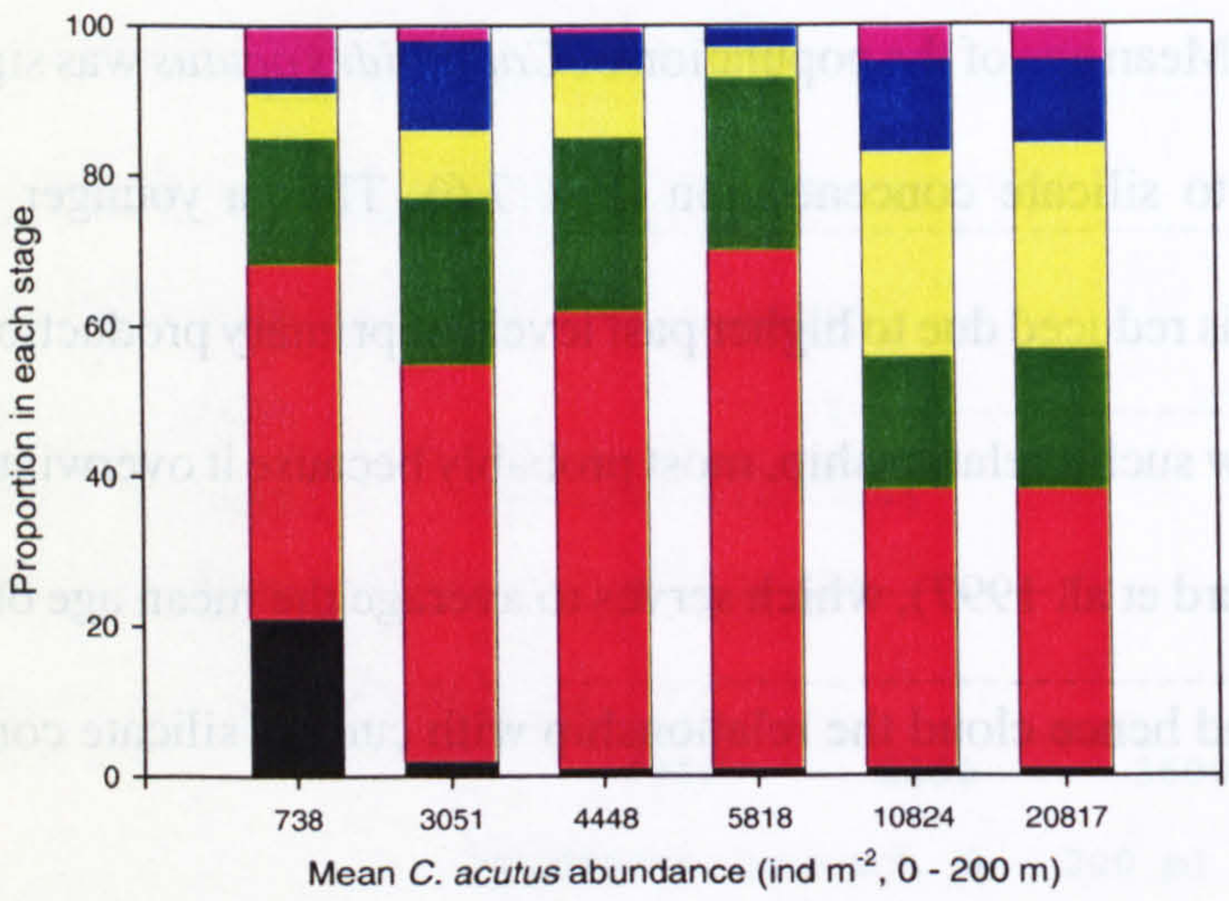


Figure 7.5 *Calanoides acutus*. A, Abundance (ind m^{-2}, 0 - 200 m) of each stage in relation to total abundance, and B, proportionate contribution of each stage to the total abundance.

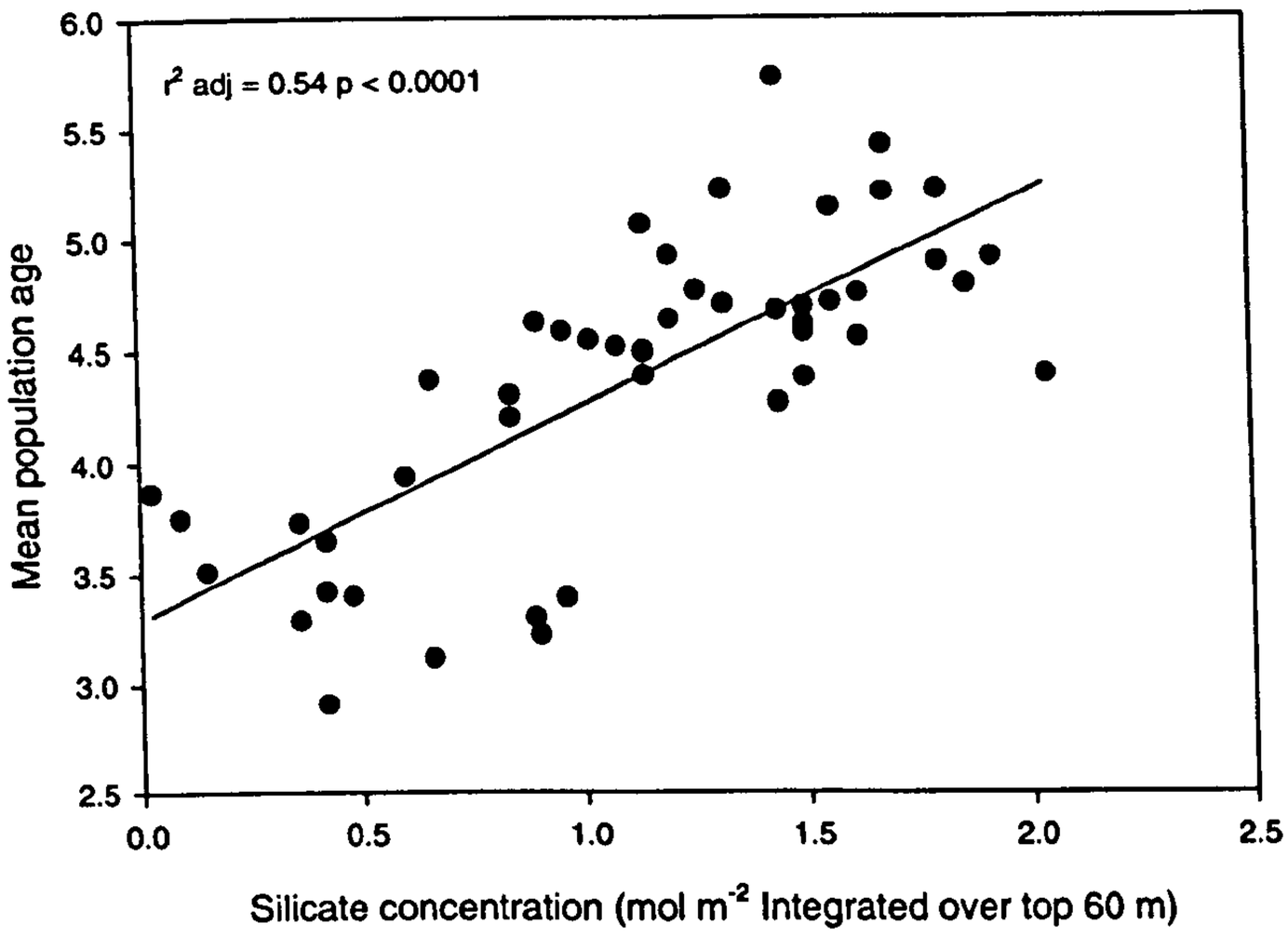
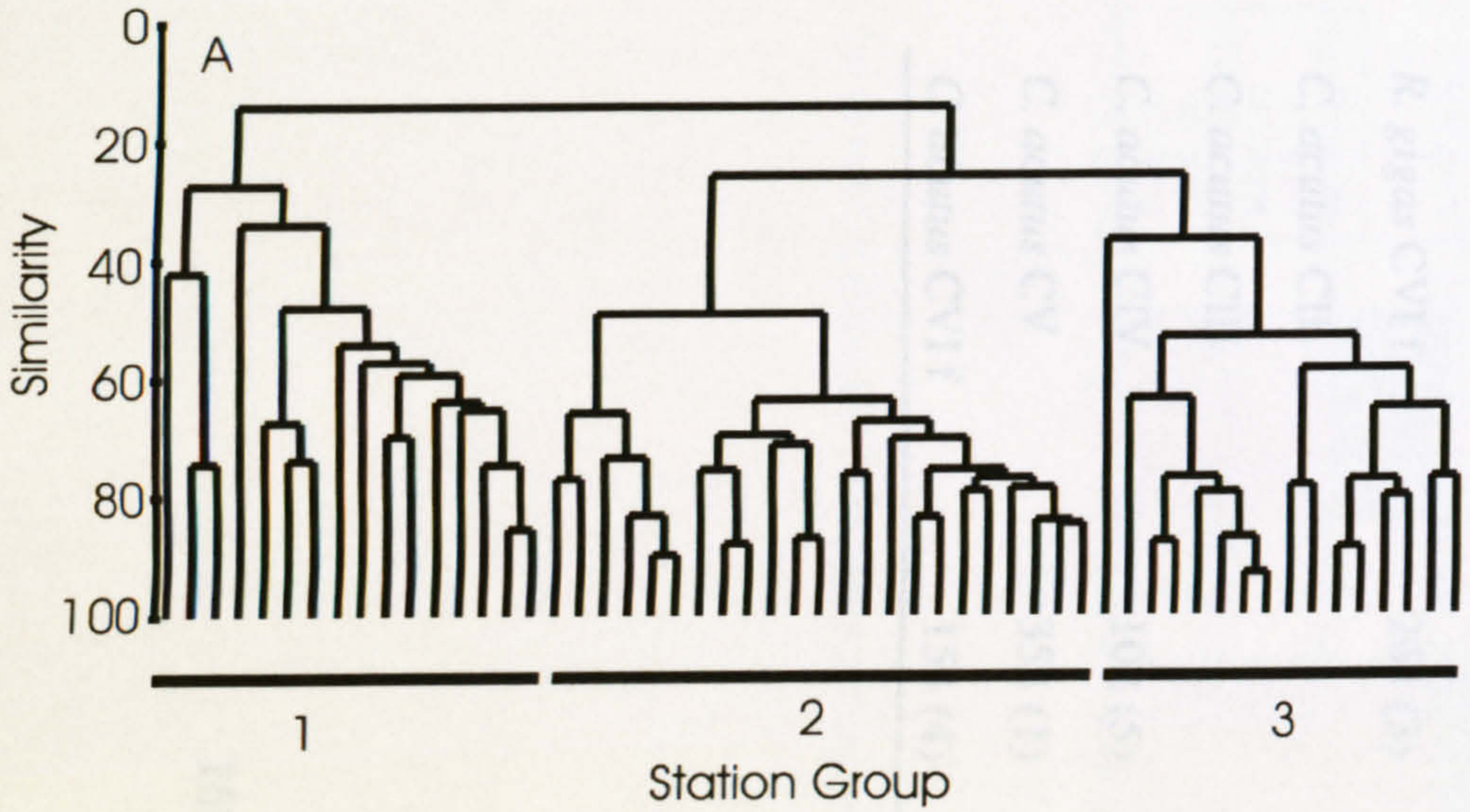


Figure 7.6 *Calanoides acutus*. Mean age of the population in relation to integrated silicate concentration (mol m^{-2}, 0 - 60 m). Solid line, least squares regression.

Cluster analysis of *Rhincalanus gigas* and *Calanoides acutus* net data across all four years revealed three main station groups separated at an arbitrary level of < 26 % similarity (Fig. 7.7A). ANOSIM indicated that there were significant ($p = 0.001$) differences between the 3 groups (Global R statistic = 0.893). Further tests using SIMPER showed that station group 1 was characterised by older copepodite stages (CIV - CVI f) of both species, group 3 by a high abundance of the younger copepodite stages

(CII - CV) of *C. acutus* and *R. gigas* nauplii, and group 2 was intermediate in abundance with a population made up of some younger stages, but lacking dominance of adult females (Table 7.4). MDS produced an ordination with low stress (0.08) indicating that the stations were well represented in 2D ordination space (Fig. 7.7B). Overall abundances of *R. gigas* and *C. acutus* in relation to the station groupings are shown in Fig. 7.7C. Data were grouped predominantly, although not exclusively, by year. The 1997/98 season was exclusively within group 2, with the incorporation of seven other stations. Stations from 1998/99 and those from off-shelf in the western mesoscale box during 1996/97 dominated group 3, and stations from 1995/96 and 1996/97 made up group 1.

Figure 7.7 A. Results of Bray-Curtis Similarity Cluster on total abundance of all copepodite stages of *Rhincalanus gigas* and *Calanoides acutus*. 'Station groups' 1 - 3 were arbitrarily designated based on a similarity level of < 34 %. B. MDS ordination of the station groups identified in the cluster analysis, group 1 (▲), group 2 (■) and group 3 (▼). C and D. Bubbleplot representations of *R. gigas* and *C. acutus* total abundance (C) and silicate concentration (D). Data have been superimposed on the 'station groups' ordination space (B) to clarify spatial relationships. The size of the bubbles are scaled to the minimum and maximum values for each variable. (*R. gigas* and *C. acutus* total abundance 185 - 66328 ind m^{-2},0 - 200 m), silicate 0.024 - 2.04 mol m^{-2}, 0 - 60 m).


A
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20
40
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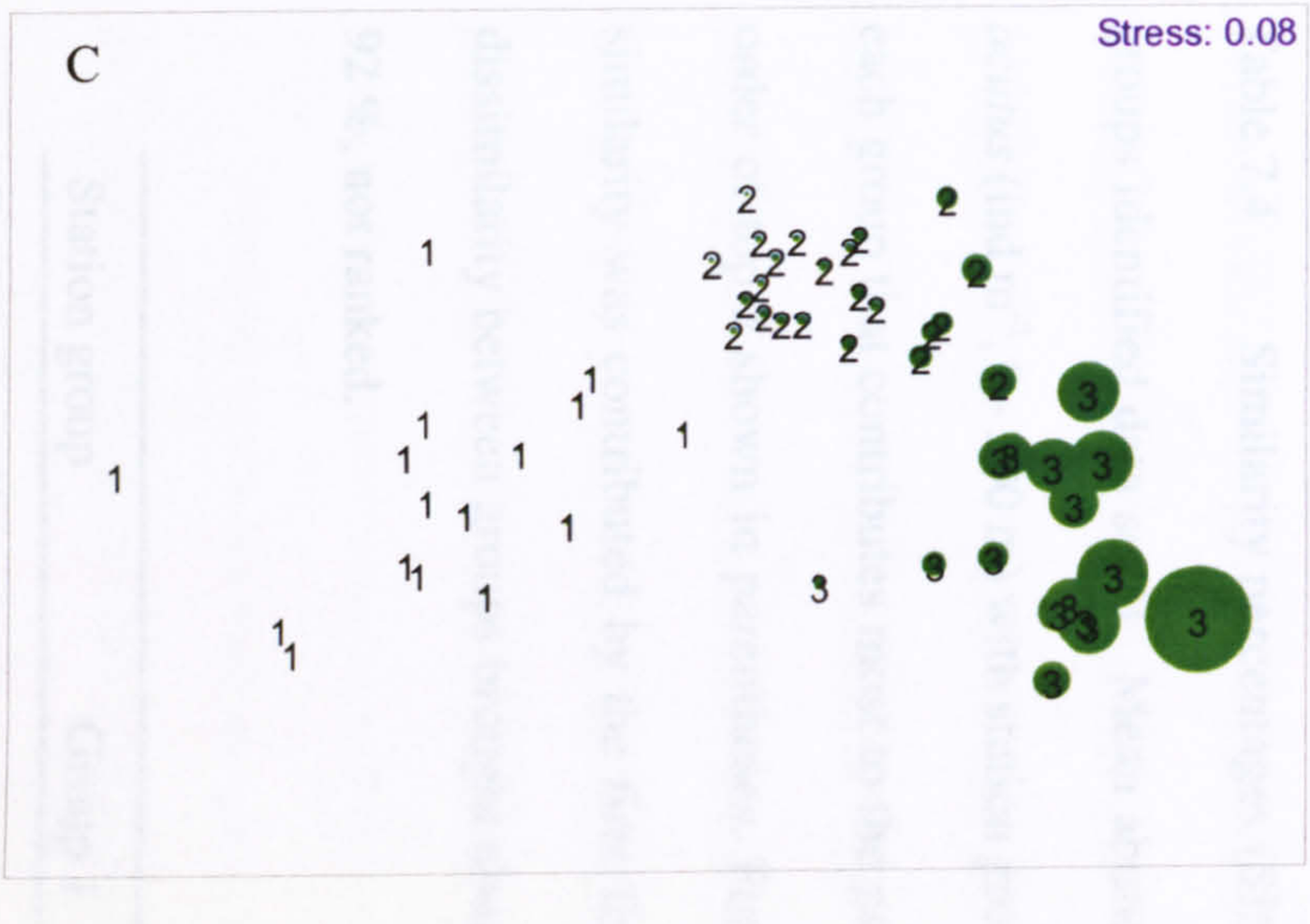
3
Station Group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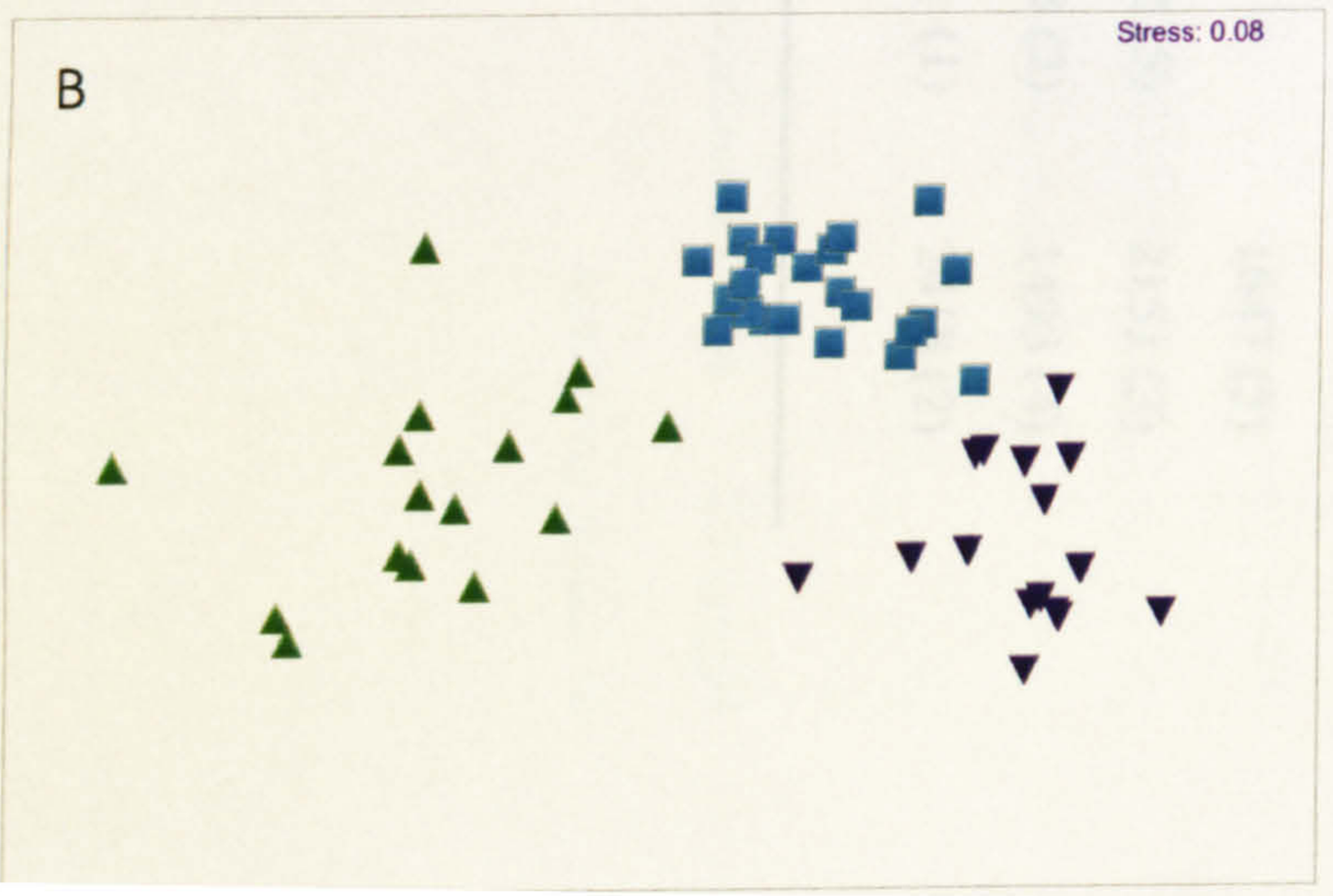
Stress: 0.08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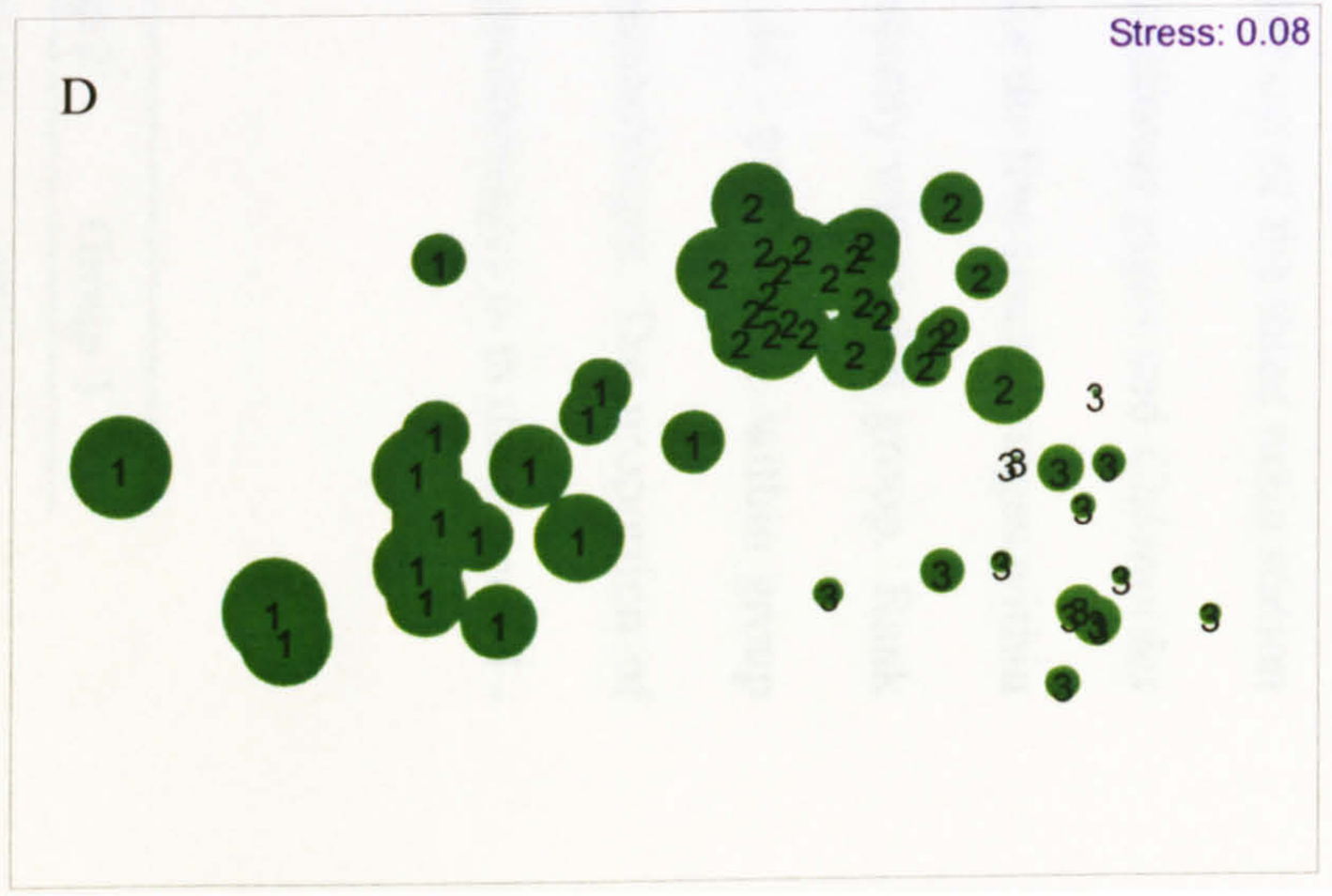

D
Stress: 0.08

Figure 7.7

Table 7.4 Similarity percentages (SIMPER), carried out of the three main station groups identified data set B. Mean abundance of *Rhincalanus gigas* and *Calanoides acutus* (ind m^{-2}, 0 - 200 m) with station groups is given for the five species stages within each group that contributes most to the percentage similarity within that group. Rank order of top 5 shown in parentheses. For each group 44 - 61 % of the within group similarity was contributed by the first five ranked species/stages. The proportion of dissimilarity between groups brought about by these species/stages is in the range 74 - 92 %, not ranked.

Station group	Group 1	Group 2	Group 3
R. gigas naupliar		1083 (3)	19778 (1)
R. gigas CII		433 (4)	
R. gigas CIV			
R. gigas CV	230 (2)		
R. gigas CVI f	263 (3)		
C. acutus CII			1647 (5)
C. acutus CIII		302 (5)	2151 (3)
C. acutus CIV	102 (5)	1438 (2)	1493 (4)
C. acutus CV	353 (1)	4256 (1)	2418 (2)
C. acutus CVI f	154 (4)		

The mean stage frequency of *C. acutus* in these three groups are shown in Fig 7.8. Group one (mean age 5.07) showed the characteristics of an old, overwintered population with little or no recruitment having occurred, suggested by the small numbers of stage CV and CVI females, and the complete lack of younger copepodite stages. In contrast group three, (mean age 3.45), had a high abundance of all copepodite stages, suggesting recruitment was currently occurring and had been taking place for some time. Group two, (mean age 4.58), which had a high abundance of stage CV and lack of younger copepodite stages, was interpreted as a population which had completed recruitment.

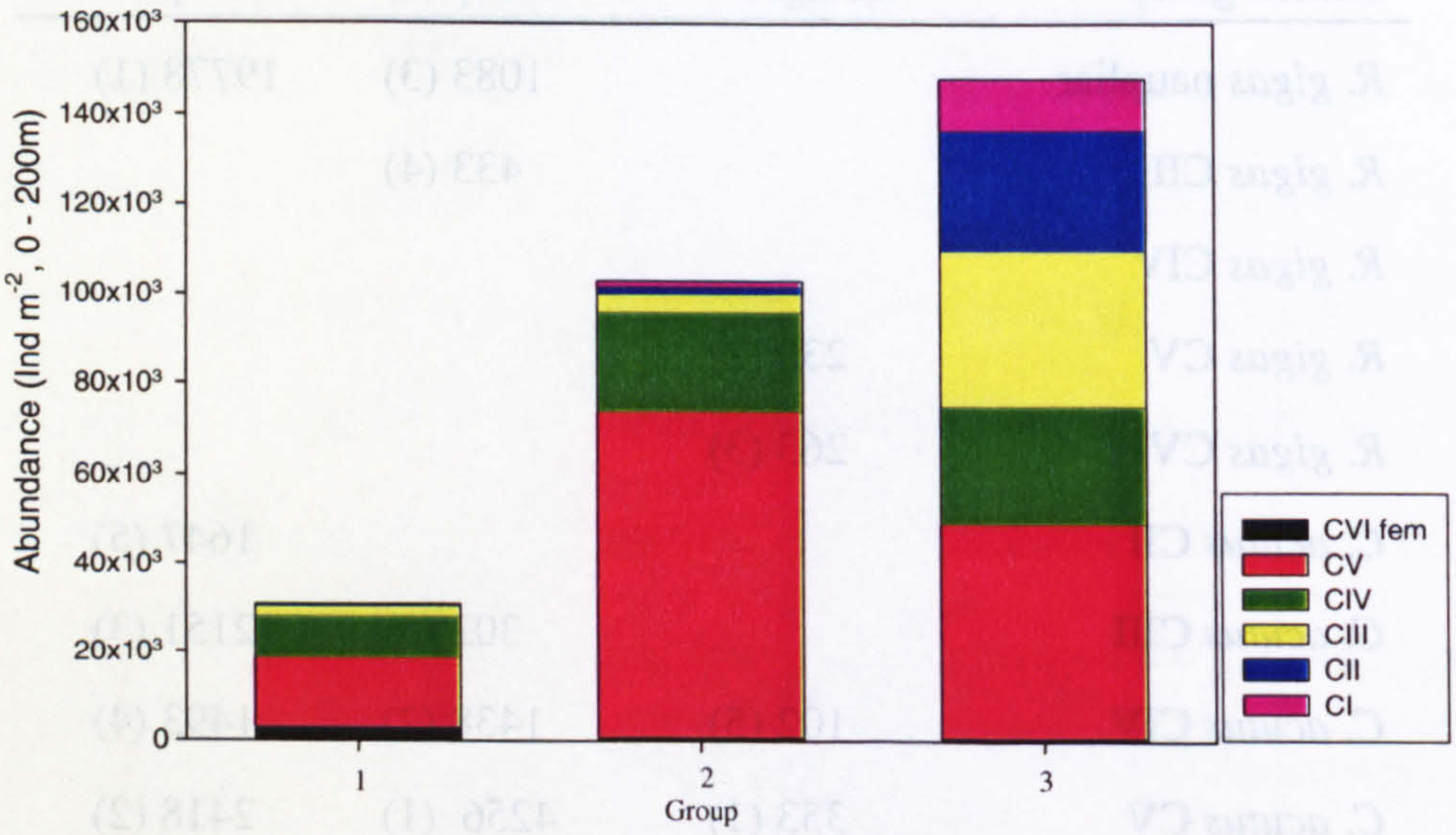


Figure 7.8 Mean abundance of each copepodite stage of *Calanoides acutus* in relation to station groupings.

Comparison of two main species with rest of copepod assemblage

Cluster analysis of data set C showed similar station groupings to those for set B (Fig 7.9 A). Thirty three out of 55 stations fell within the same 3 station groupings. ANOSIM indicated that there were significant ($p = 0.001$) differences between the three groups (Global R = 0.851). Whilst forcing the station grouping of data set B onto C reduced the Global R to 0.51, this grouping was still stronger than using either year, area or on-off shelf as a factor (0.285, 0.269 and 0.061 respectively). Therefore the station groupings of the *Rhincalanus gigas* and *Calanoides acutus* populations were a reasonable representation of the rest of the copepod assemblage.

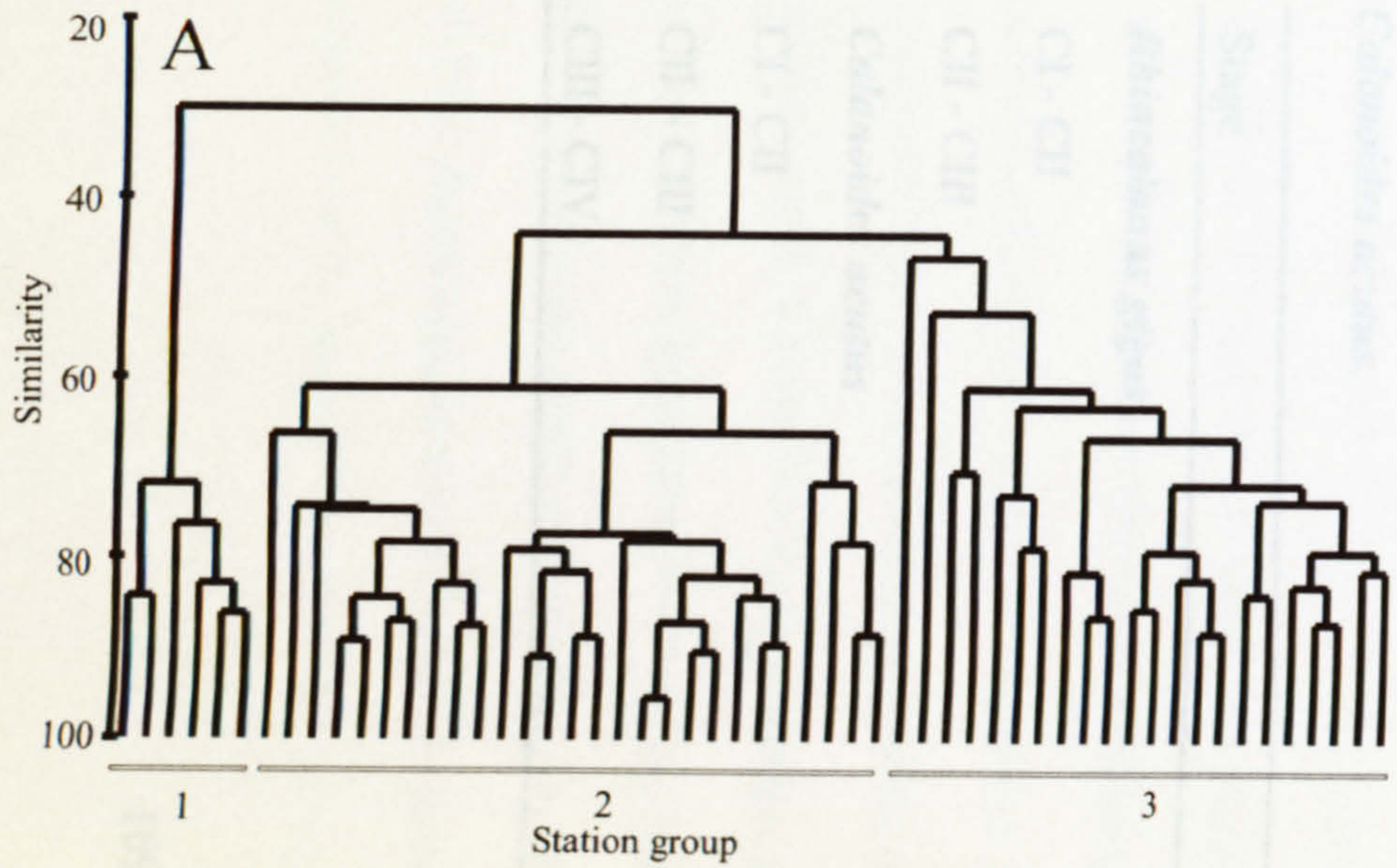
Similarity between stations groups of data set C was defined predominantly by the abundance of *Oithona* spp., *Ctenocalanus* spp. and *Metridia* spp. stages CI - CIII. (Table 7.5). MDS produced a an ordination with low stress (0 .07) indicating the stations were well represented in 2D ordination (Fig. 7.9B).Overall abundances of data set C in relation to station groupings are shown in Fig. 7.9C.

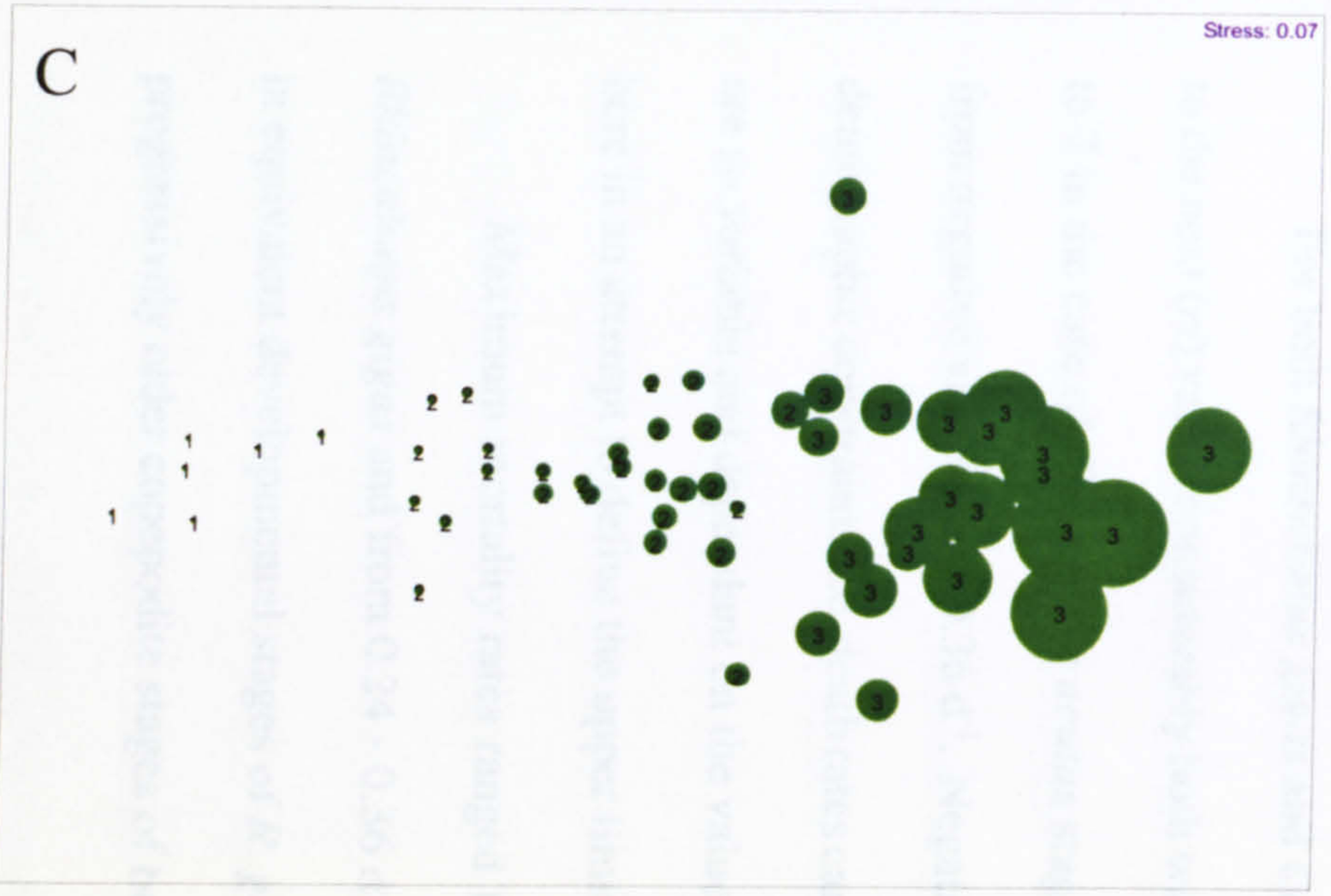
BIOENV indicated that the station groupings of data sets B and C were most closely described by just one environmental factor, silicate concentration ($\rho w = 0.402$, $\rho w = 0.381$ respectively). Silicate concentration in relation to station groupings for set B & C are shown in Figs. 7.8D & 7.9D respectively.

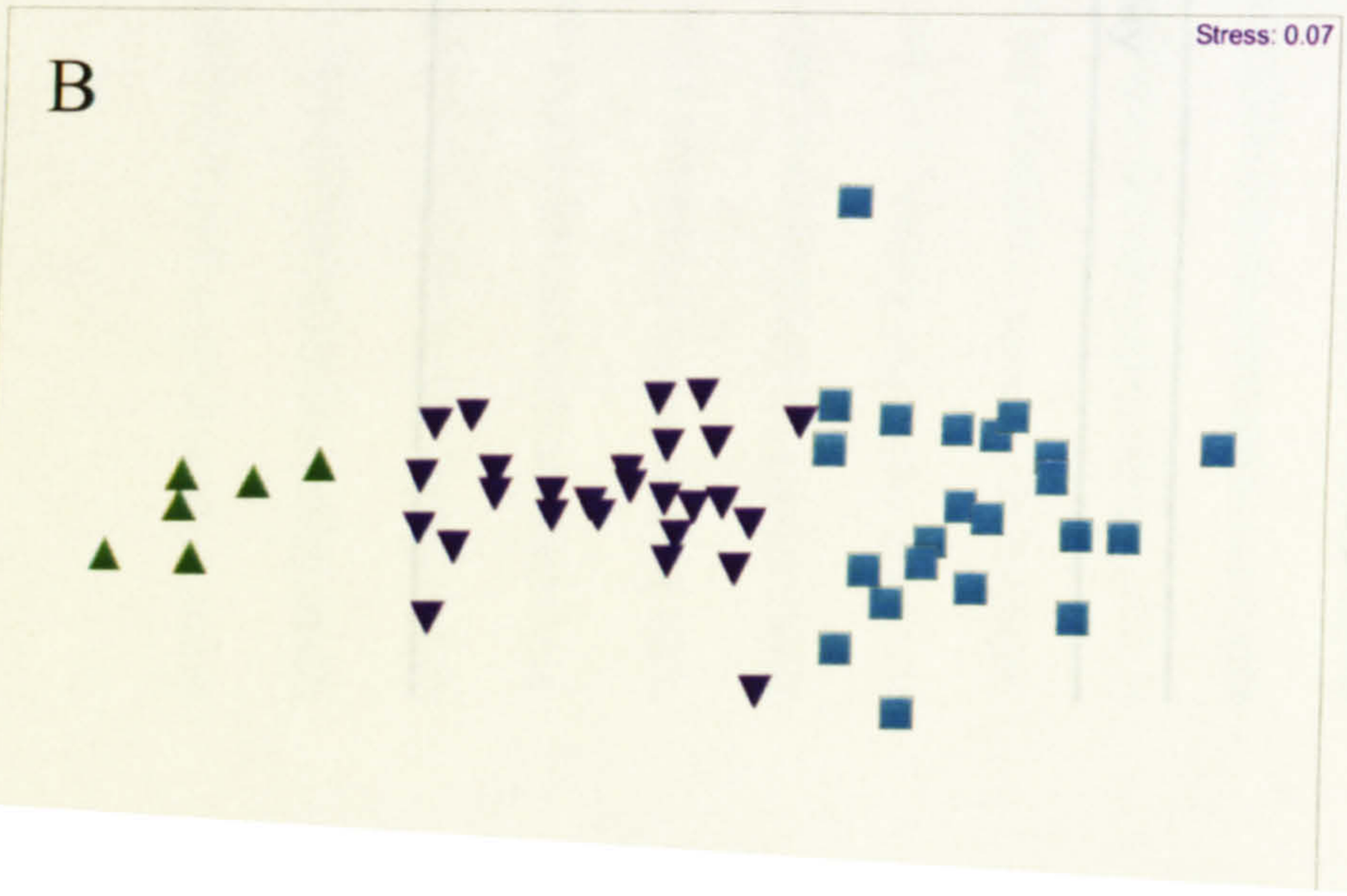
Table 7.5 Similarity percentages (SIMPER), carried out on the three station groups identified in data set C. Mean abundance (ind m^{-2}, 0 - 200 m) with station groups is given for the three species within each group that contributes most to the percentage similarity within that group. Rank order of top 3 shown in parentheses. For each group > 90% of the within group similarity was contributed by the first three ranked species. The proportion of dissimilarity between groups brought about by these species/stages is in the range 56 and 85 %, not ranked.

'Station group'	1	2	3
Oithona spp.	15787 (1)	45904 (1)	99285 (1)
Ctenocalanus spp.	1186 (2)	10217 (2)	43356 (2)
Metridia spp, CI- CIII	315 (3)	6284 (3)	28603 (3)

Figure 7.9 A. Results of Bray-Curtis Similarity Cluster on data set C. 'Station groups' 1 - 3 were arbitrarily designated based on a similarity level of < 45 %. B. MDS ordination of the station groups identified in the cluster analysis, group 1 (▲), group 2 (■) and group 3 (▼). C and D. Bubbleplot representations of total abundance of individuals in data set C (C) and silicate concentration (D). Data have been superimposed on the 'station groups' ordination space (B) to clarify spatial relationships. The size of the bubbles are scaled to the minimum and maximum values for each variable. (Individuals in data set C 13,848 - 502,445; silicate 0.024 - 2.04 mol m^{-2}, 0 - 60 m).


A
20
40
60
80
100
Similarity
1
2
3
Station group


C
Stress: 0.07


B
Stress: 0.07

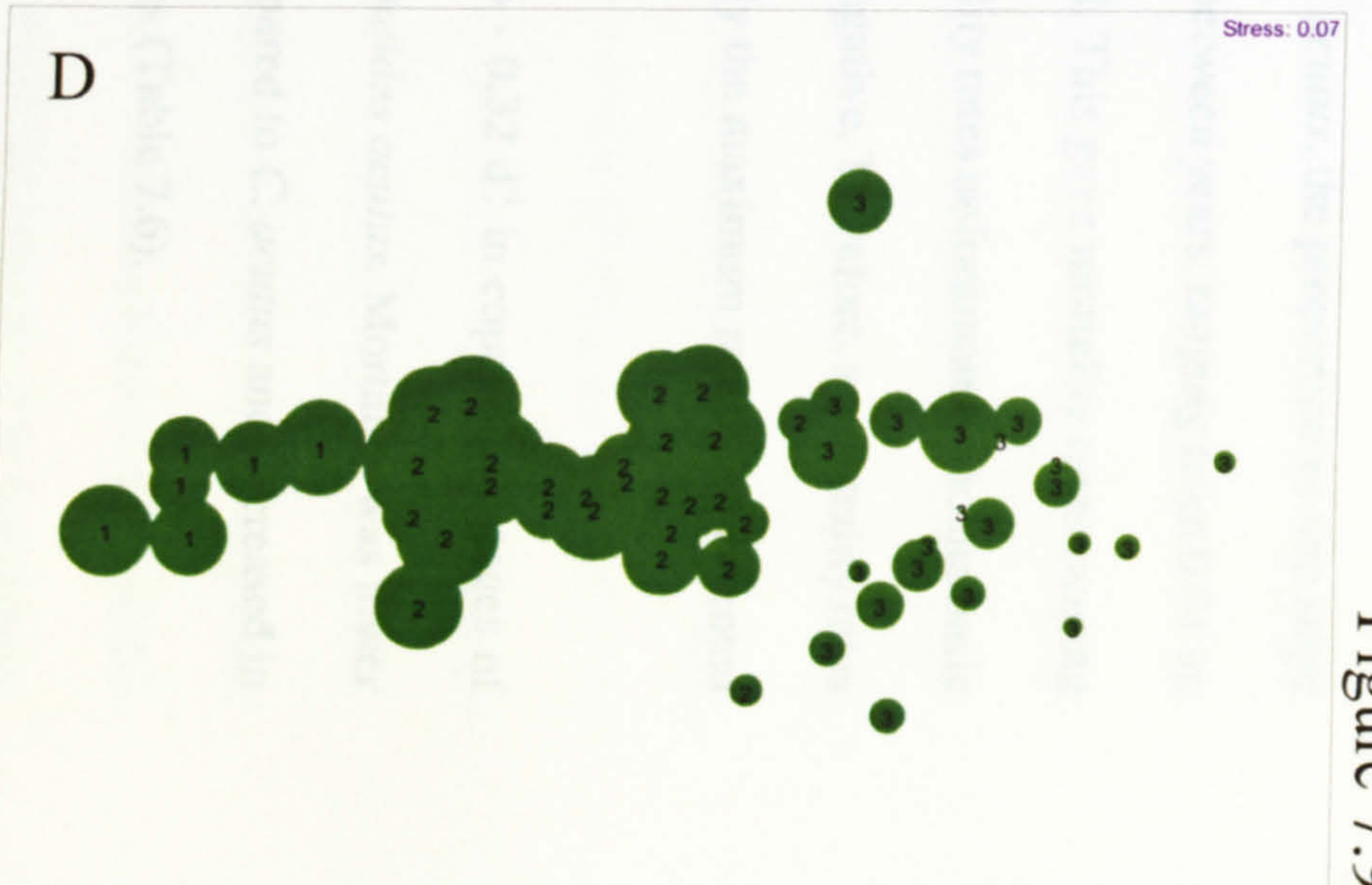

D
Stress: 0.07

Figure 7.9

Mortality rates

For both *Rhincalanus gigas* and *Calanoides acutus*, the proportion of one stage to the next (*ri*) varied considerably both within and between years, ranging from 0.01 up to 7 in the case of *Calanoides acutus* stage CI - CII. This gave mortality rates ranging from negative values up to 0.36 d^{-1}. Negative mortality rates unfortunately violate basic demographic constraints, i.e. death rates can not be negative. Therefore, as mortality rates are so variable and dependant on the value of *ri*, only the maximum rates are presented here in an attempt to define the upper limits.

Maximum mortality rates ranged from 0.26 - 0.32 d^{-1} in copepodite stages of *Rhincalanus gigas* and from 0.24 - 0.36 d^{-1} in *Calanoides acutus*. Mortality was lower in equivalent developmental stages of *R. gigas* compared to *C. acutus* and decreased in progressively older copepodite stages of both species (Table 7.6).

Table 7.6 Maximum mortality (d^{-1}) rates estimated for *Rhincalanus gigas* and *Calanoides acutus*.

Stage	Mortality
Rhincalanus gigas	
CI - CII	0.32
CII - CIII	0.26
Calanoides acutus	
CI - CII	0.36
CII - CIII	0.34
CIII - CIV	0.24

7.4 Discussion

Copepod abundance

Although copepod populations were essentially sampled during the same part of the season each year (mid December - mid January), abundance varied considerably (approximately 30 fold). Increased total copepod abundance was due to an increase in abundance of all species already present, whilst maintaining their relative proportions, and was significantly and negatively related to the past feeding environment.

Detailed study of the two key species revealed that, whilst total numbers of individuals increased, female abundance did not differ significantly between years. This, combined with the facts that the mean age of the population of *C. acutus* decreased while total population abundance increased, suggests higher recruitment, rather than the retention and concentration of the population. The relationship between mean age of the population of *C. acutus* and silicate concentration (Fig. 7.5) imply that the recruitment of this species is dependant on the past feeding environment, and therefore population structure is most likely controlled by fecundity.

As fecundity is tied to primary production (see Fig. 6.1), it suggests that where copepod abundance is highest, the standing stock of chlorophyll must have remained in the order of $\geq$ 3 mg chl m^{-3} (0 - 60 m), for a significant period in order to support higher levels of egg production (see Chapter 6, critical food concentrations for egg production). This idea is supported by the high level of silicate depletion in areas of high copepod abundance and the occurrence of the complete range of younger copepodite stages of the two key species. The station groupings observed in the *Rhincalanus gigas* and *Calanoides acutus* generally reflected those of the whole copepod assemblage.

The degree of silicate depletion may be used to estimate the timing of the initiation of the bloom. Silicate levels around South Georgia are typically around 30 mmol m^{-3} prior to a bloom (equivalent to 1.8 mol m^{-2}, 0 - 60 m) (Whitehouse et al. 1993, 1996). This level of silicate was recorded at some stations during this survey, (see Chapter 3, Fig. 3.8) and it was assumed that here phytoplankton growth had been negligible. Silicate depletion during the course of this study was recorded as low as 0.25 mol m^{-2}, (0 - 60 m), and from this an estimate of the amount of carbon fixed in primary production has been made. A C:Si molar ratio of 4, measured on the phytoplankton assemblage around South Georgia by Priddle et al. (1995) was applied, to give an estimate of the amount of carbon fixed in this way. It was estimated that 6.2 mol C m^{-2}, equivalent to 74 g C m^{-2}, was fixed since production started. The time scale required for this amount of carbon fixation, assuming a daily rate of carbon fixation of 1.2 g C m^{-2}, measured by Owens et al. (1991) in waters to the NE of South Georgia, was found to be in the order of 60 days, placing the onset of the bloom to around mid October. Whilst this approach serves to estimate the probable longevity of the bloom, the method assumes that phytoplankton growth rates are linear throughout, and that the value used is representative, and no correction for grazing has been included.

This time scale was supported by the high copepod abundance found in silicate depleted areas, along with the occurrence of all copepodite stages of *Calanoides acutus*, suggesting that recruitment had been continual over a period approximating to 2 - 3 months. This time period was estimated bearing in mind that, in the region in which this study took place, *C. acutus* is thought to overwinter in stages CIV and CV, which moult to adults during late winter. Therefore the younger population that is present must result

from the current years recruitment. An estimate of the amount of time that *C. acutus* may take to develop from egg to stage CV at a mean water temperature of 2.5°C can be made by combining the development times measured during this study (Chapter 4), with naupliar development times derived using the concept of equiproportional development. This method assumes that each developmental stage occupies the same proportion of time relative to egg development at any given temperature (see Fig 4.1 for details). Whilst the later copepodite stages of *C. acutus* do not appear to conform to this mode of development, it is anticipated that this method will give a sensible estimate of naupliar development time. Development of naupliar stages i - vi takes approximately seven times that of embryonic duration (derived from Thompson 1982). By multiplying the egg hatching times of 4.75 d derived for *C. acutus* at 2.5°C by Ward & Shreeve (1998) by seven, the total naupliar development time is estimated to be in the order of 33 days. This in conjunction with the development times measured for stages CI - CIV (also 33 days), gives total development time from egg to stage CIV as about 2 months. Taken that this study took place between mid December - mid January, this then places the onset of egg production to mid October - mid November in this part of its range. This is in agreement with observations made on the population of *Calanoides acutus* by Andrews (1966) in a similar geographical region, lying between the most northerly extent of the ice-edge and the Antarctic Convergence (ie Polar Front) (see Mackintosh & Herdman 1940. pl LXIX). During October, female *C. acutus* started appearing in the plankton which Andrews (1966) considered to be in egg laying condition.

Whilst the younger, actively recruiting population of *C. acutus* was found associated with silicate depleted areas, and the oldest population in which no recruitment had occurred was associated with silicate replete areas, station grouping two, which was

interpreted as a population where recruitment had been completed was associated with an intermediate silicate concentration of around 1.25 mol m^{-2}, 0 - 60 m (Fig. 7.8). This group was formed of stations undertaken slightly later in the year than the other two groups, when *C. acutus* may be at the end of its recruiting phase, and major episodes in phytoplankton growth may be over. The intermediate concentration of silicate suggests that phytoplankton growth may have been sub-optimal compared to levels observed in station grouping 3, and the lower abundance of both *R. gigas* and *C. acutus* in group 2 suggest lower recruitment.

The strong relationships of both the total copepod population and the stage frequency of *Calanoides acutus*, with silicate concentration reflects the importance of the timing and magnitude of primary production to copepod population dynamics. The relationships found in this study may be used to infer more about the timing and duration of phytoplankton blooms in the South Georgia area in the future.

Mortality

The 'vertical life table approach' used to estimate mortality in this study is sensitive to the proportion of one stage to the next (*ri*). In this study *ri* ranged widely both within and between the four years studied, consequently mortality rates ranged from negative values up to 0.36 d^{-1}. Population stage frequencies of both *Rhincalanus gigas* and *Calanoides acutus* were however all taken within a 6 week period during the summer, during which time their recruitment is thought to be continuous (Marin 1988). The wide range in the value of *ri* may be seen in the populations for one of two reasons. Firstly the onset of reproduction and fluctuations within it will vary depending on factors such as the timing and longevity of the phytoplankton bloom, this may vary both spatially

and temporally, and hence effect the timing of recruitment into a stage. Secondly, older individuals from a previous generation may have over wintered and hence may serve to elevate the numbers in one stage compared to another. Aknes & Ohman (1996) cautioned the interpretation of mortality rates when *ri* showed high variance. Whilst estimates of mortality rates in this study are therefore limited, they are the first rates to be presented for these species during summer months.

Rates for both species decreased with increasing stage, and were higher in *C. acutus* than in equivalent stages of *R. gigas*. Maximum rates of mortality during summer for stages CI - CIII *Rhincalanus gigas* (mean 0.29), and CI - CIV *Calanoides acutus* (mean 0.31) are two orders of magnitude higher than those estimated for the whole populations during autumn/winter. Mortality estimates during the autumn/winter period, when the population is diapausing at depth, were in the order of < 0.004 d^{-1} for *R. gigas* and 0.007 d^{-1} for *C. acutus* (Ward et al. 1997, Atkinson et al. 1997 respectively).

The only comparable estimates of mortality in calanoid copepods during summer are for the latter four copepodite stages of *C. finmarchicus* and *C. hyperboreus*, where mortality rates from 0.02 - 0.4 and 0.003 - 0.007 d^{-1} were reported for each species respectively, from Korsfjord, western Norway (Matthews et al. 1978). Mortality rates measured in this study therefore compare more closely to the higher rates measured for *Calanus finmarchicus*.

In the final chapter the copepod population dynamics are discussed in relation to the South Georgia ecosystem, and areas for future research outlined.

Chapter 8 The marine ecosystem around South Georgia

Overview

This study has provided the first instantaneous measurements of the growth and development of *Rhincalanus gigas* and *Calanoides acutus*, in relation to concurrently measured environmental factors. It has highlighted the variability in their biomass, and demonstrated clear relationships with environmental variables, particularly silicate. The strong relationships of both the total copepod population and the mean age of *C. acutus*, with silicate concentration reflects the importance of the timing and magnitude of primary production to copepod population dynamics around South Georgia. The data and relationships found in this study may be used to model more accurately the population dynamics in these two species, and in the interpretation of the timing, duration and fate of phytoplankton blooms in the South Georgia region.

The inverse relationship found between copepod abundance and krill (*Euphausia superba*) biomass at the meso and inter-annual scale appears to be driven by physical factors rather than direct competition between the two groups. This in is contrast with a previous study in the region by Atkinson et al. (1999) who also demonstrated an inverse relationship at the fine and mesoscale. They suggested that this was likely due to predation of copepods by krill, although did not rule out the possibility of differences in the physical environment or of the timing of the phytoplankton bloom as being more important particularly at the inter-annual scale. Whether plankton communities are controlled mainly by bottom up or top down control, depends strongly on the scale at

which the problem is considered. At large scales, copepod communities tend to be controlled from below, whilst on small scales population dynamics may be influenced more by predation pressure (Kiørboe 1998). In this study, with a stronger inter-annual theme compared to that of Atkinson et al. (1999) a stronger relationship was found between total copepod abundance and silicate than with krill, leading to the view that over the four years, the environment and the timing of the bloom, rather than krill were the more important factors. The facts that egg production rates of both *Rhincalanus gigas* and *Calanoides acutus* were food limited over much of the survey period and the abundance of their younger copepodite stages and the mean age of the *C. acutus* population, were all strongly related to silicate also supports this view. Results of this study therefore suggest that copepod populations around South Georgia appear to be controlled more significantly by bottom-up mechanisms, rather than top-down.

Whilst these data only begin to help describe the complex relationships in the South Georgia marine ecosystem, they have allowed the assignment of parameters to key areas of copepod growth and development which are essential if the population dynamics of these Antarctic species are to be modelled.

Food sources

Of the environmental factors considered in this study, it was the concentration of silicate that best reflected the patterns in copepod abundance and body carbon mass. Higher copepod abundance and biomass was associated with areas of greater silicate depletion, which is a proxy for the degree of diatom production in the system since the beginning of the current growing season. In Chapter 6 (Fig 6.1) egg production was observed to increase with higher chlorophyll concentrations, and in Chapter 7, it is

concluded that recruitment into the copepod population was higher when there had been higher levels of diatom growth. This suggests that hatching viability had not been seriously impaired by the presence of high concentrations of diatoms. Hence the suggestion concerning the insidious effects of diatoms on copepod reproduction and hatching viability (Ianora et al. 1996, Miralto et al. 1999) are not supported by this current data set. More recent literature on this debate also support the idea that diatoms do not have any negative effect on egg production or viability (Irigoien et al. 2000, in press).

Whilst the level of silicate depletion was the best predictor of those tested in this study, it explained only 58 % of the variance in copepod abundance. Whilst it would be anticipated that no one single factor could explain all the variation, it may be that in this case other factors, which were not measured in this study, are as important as the past levels of diatoms. A review by Garrison (1991) of the abundance and role of Antarctic protozoans suggests that heterotrophic flagellates (dinoflagellates and other heterotrophic nanoplankton) and ciliates (mostly non-loricate oligotrichs) dominate in the surface waters, and may play an important role in the Southern Ocean food web dynamics. During feeding experiments within the Atlantic sector of the Southern Ocean, *Rhincalanus gigas* and *Calanoides acutus* fed on an ambient food supply were shown to ingest micro-flagellates and large diatoms in an approximately 1:2 ratio. However when the food supply was enriched, the frequency at which diatoms were taken increased (Schnack 1985). Similar feeding experiments conducted around South Georgia, showed that whilst *R. gigas* and *C. acutus* showed a preference for large diatoms, motile taxa did account for a proportion of their diet (Atkinson et al. 1996). Further studies need to address the importance of protozoans within the ecosystem around South Georgia and

their relevance to copepod growth.

Biomass and production estimates

The importance of copepods in the Southern Ocean food web has recently been highlighted by Voronina (1998). She compared the biomass and annual production of three key filter-feeding groups of zooplankton; krill, salps and four species of copepod; *Rhincalanus gigas*, *Calanoides acutus*, *Calanus proinquus* and *Metridia gerlachei* across the whole of the Antarctic pelagic zone. Production and biomass estimates indicated that in terms of fresh mass, the main proportion of filter feeding biomass was attributable to salps, with copepods and krill laying in second and third places respectively. When these estimates were recalculated in terms of dry mass, copepods comprised 45% of the filter feeding biomass, with krill and salps forming 36% and 19% respectively. The data collected during the present study was used to estimate the relative production of the two species of copepod and was compared to estimates made for krill using literature values.

The mean and the range of biomass were calculated for the populations of *Rhincalanus gigas* and *Calanoides acutus*, using the station specific abundance and carbon mass data determined in this study (see Chapters 7 and 5 respectively) and are presented in Table 8.1. Production for each species (g C m^{-2}, 0 - 200 m) was calculated as a function of their biomass multiplied by their mean mass specific growth rates (g). The latter was calculated by taking the average of the stage specific g presented in Table 6.1, 0.11 for *C. acutus* and 0.04 for *R. gigas*. Krill biomass estimates were taken from Brierley et al. (1997). Their wet mass is converted to carbon mass by first assuming dry mass is 75 % of wet mass (Morris et al. 1988), and that carbon mass was 40 % of dry

mass (Schnack 1985, Ikeda & Kirkwood 1989). Production was estimated by using two values from the literature which have been quoted for mass specific growth rate of krill in the South Georgia region. From an intensive 6 day study around South Georgia, the modal length of krill within a swarm increased by 2 mm (Clarke & Morris 1983), equivalent to a daily mass specific growth rate of ~3.5 % body mass d^{-1} (Atkinson et al. 2001). Rosenberg et al. (1986) suggested a lower value of ~2% body mass d^{-1} based on the mean monthly change in length compositions of krill stocks measured during the Discovery expeditions between the years 1928 - 1938. The two estimates of production for krill are shown in Table 8.1 as two values for the mean, minimum and maximum.

Table 8.1 Standing stock of biomass (g C m^{-2}, 0 - 200 m) and daily production (g C m^{-2} d^{-1}, 0 - 200 m) of the populations of *Calanoides acutus* and *Rhincalanus gigas* estimated from data presented in this study, and for *Euphausia superba* estimated from literature values (see text for details).

	Biomass			Production		
	Mean	Min	Max	Mean	Min	Max
C. acutus	0.55	7.13^{-03}	1.80	0.06	7.1^{-04}	0.18
R. gigas	0.71	1.89^{-04}	6.34	0.03	7.0^{-06}	0.23
E. superba	4.40	0.19	15.10	0.09 - 0.15	3.7^{-03} - 6.6^{-03}	0.30 - 0.53

The biomass of *Rhincalanus gigas* was higher than that of *Calanoides acutus*, whilst daily production was higher in *C. acutus*, because of its higher mass specific growth rate. Biomass of the two species of copepod were individually both lower than for krill, but their combined production rates approximated that of krill. This supports one

view of food web dynamics in the Southern Ocean, that total copepod production may be equal to, or in excess of, that of *Euphausia superba* (Boysen-Ennen et al. 1991, Conover & Huntley 1991, Voronina 1998). Therefore copepods probably play an important role in the flux of local production in the South Georgia ecosystem.

Production to Biomass ratio. Comparison with northern hemisphere

Measurements made by Ikeda (1985) on the metabolic rates of epiplanktonic communities showed that between 85 - 95% of the variation can be attributed to body size and environmental temperature. These measurements were based on a wide range of epipelagic communities, each comprising about 50 - 150 species ranging over six orders of magnitude, and occurring in various latitudes with environmental temperatures ranging from -1 - + 30°C. Ikeda (1985) had two principle findings: firstly a strong negative correlation between adult body mass and water temperature, and secondly a strong positive correlation between body size and metabolism. It would therefore be expected that copepod production and biomass would be similar at higher latitudes in both hemispheres, because they both experience similar, low, environmental temperatures.

In the literature, production is most commonly compared between populations using daily production to biomass ratios (P:B), which are in equivalent units to mass specific growth rates (g) (presented in Chapter 6). Literature values of daily P:B for congeners in the northern hemisphere, *Calanus finmarchicus*, *C. glacialis* and *C. hyperboreus* range from 0.008 - 0.37 (Tremblay & Roff 1983, Aksnes & Magnesen 1988), with values of g for *Calanoides acutus* and *Rhincalanus gigas* presented in the current study ranging from 0.005 - 0.24. These measurements suggest that daily

production of the biomass dominant copepods in the Southern Ocean are of a similar order of magnitude to congeners in the northern hemisphere, and fall within the general rules for epipelagic plankton proposed by Ikeda (1985).

Role of copepods in the Southern Ocean food web

Whilst this study highlights the importance of copepods in the South Georgia ecosystem, more research needs to be directed to quantify their position in the food web. In the northern hemisphere, copepods are very important as a food for fish, especially for pelagic fish (Marshall & Orr 1972, and references therein). It is this direct link to the fishery that has stimulated much of the research into copepods in the northern hemisphere. Indeed international programs have been conducted, such as the Trans Atlantic Study of *Calanus* (TASC), whose remit was to look predominantly at the biology and population dynamics of just one species, *Calanus finmarchicus,* in the North Atlantic.

There are only a limited number of studies which place Southern Ocean copepods in the context of the food web, but they are clearly important at a number of trophic levels. Calanoid copepods are of prime importance in the marine ecosystem because many are herbivorous and form one of the main links between primary production and higher trophic levels. At South Georgia grazing impact by copepods on the primary production has been estimated to be highly variable dependant on the time of sampling. A study conducted during a spring phytoplankton bloom at South Georgia showed that less than 5% of daily primary production was removed per day by the numerically dominant copepods (Atkinson et al.1996). It was demonstrated that 20% of this chl *a* was grazed by copepods which occurred below the mixed layer and which did not undergo

diel vertical migration. Thus this represents a potentially direct export of carbon from the system via the sinking of faecal pellets. In contrast a study during late austral summer near South Georgia by Pakhomov et al. (1997) estimated that grazing by the zooplankton community accounted for between 0.5 and 8.3% of the chl *a* biomass, which was equivalent to 5 - 102% of the total primary production, and copepods accounted for 51 - 91% of this.

Copepods are consumed in turn by organisms occupying higher trophic levels. It has been documented in the literature that small herbivorous copepods may form a major part of the diet of larger carnivorous species of copepod, such as *Euchaeta antarctica*, and may suffer high predation pressure from these species (Øresland & Ward 1993). It has also been suggested that chaetognaths may have a high predatory impact on Antarctic copepods during the austral winter (Øresland 1990). Studies conducted in the Gerlache Strait on *Eukrohnia hamata*, which comprised 94% of the chaetognaths by number, showed that most of its diet was comprised of the copepods *Euchaeta* spp., *Calanoides acutus*, *Metridia gerlachei*, *Microcalanus pygmaeus*, *Oncaea* spp, and *Oithona* spp. It was suggested that although the daily predation impact was low, *E. hamata* may have an important cumulative effect on copepod populations during the long winter period, when prey production is minimal. Similar studies on the chaetognaths *E. hamata* and *Sagitta gazellae*, in the vicinity of Marion Island (Southern Ocean) (Froneman et al. 1998), suggested that chaetognaths may predate between 0.3 and 1.2 % of the copepod standing stock, with *Oithona* spp, *Calanus* spp, and *Rhincalanus gigas* being the main prey species taken.

There is also evidence to suggest that carbon fixed within the surface waters may be directly transferred to the benthos, via copepods, within a year (Dearborn et al. 1986).

They conducted a study on the diet of the brittle star *Astrotoma agassizii* found on the Antarctic shelf both at South Georgia and along the Antarctic Peninsula. They showed that the copepods *Calanoides acutus* and *Euchaeta antarctica* were an important component of the diet of *A. agassizzii* constituting about 80% of the stomach contents.

Attempts have also been made to quantify the impact on copepod populations by the feeding populations of breeding birds at South Georgia, such as the Antarctic prion (*Pachyptila desolata*), and common diving petrel (*Pelecanoides urinatrix exsul*). Together it was estimated that they may consume annually up to one million tonnes of copepods, of which *Rhincalanus gigas* and *Calanoides acutus* make up the greatest proportion (Croxall & Prince 1987). Copepods have also been shown to be important in the diet of larval fish in the South Georgia region. A survey of five species of Antarctic fish in a fjord at South Georgia, which included three species important in the fishery; *Champsocephalus gunnari, Chaenocephalus aceratus* and *Notothenia gibberifrons*, and two other non-commercial species *Nototheniops nudifrons* and *Nototheniops larseni* found that copepods and their eggs accounted for > 90 % of all prey items in their guts (North & Ward 1990). In particular they found that the small neritic clausocalanid *Drepanopus forcipatus* comprised > 97 % of all identified copepods in the guts of *C. gunnari*, *C. aceratus* and N. *nudifrons* during winter and 97 and 87 % in *C. gunnari* and *N. gibberifrons* respectively, during summer. Copepod eggs were found in significant numbers in the guts of the larval fish, and whilst it was assumed that the eggs were ingested along with the adult females, it still represents a high mortality rate at this stage in the copepods life cycle. The demonstrated importance of small clausocalanid copepods in the diets of larval fish, may suggest that larger species of copepod may also form a significant part of the diet of fish at a later stage in their development. Indeed

observation made during the austral summer1998/99 on the stomach contents of larval *Gobionotothen gibberifrons* found in shelf waters around South Georgia, showed that the dominant prey item was stage CV *Calanoides acutus* (Shreeve pers. obs.).

Future work should be directed towards quantifying the role of copepods in the diets of commercially important fish in the South Georgia ecosystem and establishing the significance of copepods in maintaining recruitment of key fish species into the fisheries. As recruitment into the copepod populations is affected by variation in the timing and magnitude of the phytoplankton bloom, then fish recruitment may also be affected.

The way forward

In the Northern hemisphere programmes such as the Transatlantic study of *Calanus* (TASC) have supported weathership facilities in the Norwegian Sea, allowing almost daily sampling from one station for nearly two and a half months. Such sampling has provided high resolution data allowing day to day variability in abundance and stage composition to be determined (Hirche et al. 2001) and reproductive biology to be studied in more detail (Niehoff et al. 1999). In the Southern Ocean such high resolution sampling is hindered by logistical constraints, and as such, more detailed information on the life cycles, physiology, feeding and development have only started to appear in the literature in the past 10 - 15 years, and these are often based on limited data. While it may be logistically difficult to maintain weatherships in the Southern Ocean, moored sediment traps could provide data remotely, providing valuable information on the timing and composition of phytoplankton blooms, and quantify the flux of primary production through copepods. Whilst often restricted by cloud cover, images from the SeaWiFS satellite may also provide insight into the timing and longevity of surface phytoplankton

blooms.

While this thesis provides valuable information on the development time and the mass specific growth rates of the younger copepodite stages, and estimates of the variability in the abundance and biomass of two key species, there are still large gaps in the literature when compared to Northern hemisphere counterparts. As elsewhere more information is required on the stage specific mortality rates as well as estimates of egg mortality. Such estimates have been made by Peterson & Kimmerer (1994), who calculated egg mortality as a function of egg production rates, female and egg abundance in the plankton and egg development time. They demonstrated that egg mortality may be as high as 90% for *Temora longicornis* in Long Island Sound. In this respect further research could be focussed on the rates of mortality of the eggs of *Rhincalanus gigas* and *Calanoides acutus*. Other areas that warrant further research would include determination of the critical mass that a female must attain before assimilated energy can be used to produce eggs, the spawning frequency of individual females, and an estimate of how long they are reproductively active. With more such detailed information it may be possible to develop physiological models of the growth and egg production of these Southern Ocean species, such as those developed for *Calanus finmarchicus* by Carlotti & Hirche (1997).

Concluding remarks

The data presented in this study have allowed the assignment of parameters to key areas of copepod growth and development in the Southern Ocean, which should now allow the development of models of the population dynamics of these

two key species. Mathematical models have been developed for the population dynamics of *Euterpina acutifrons* in the Ligurian Sea (Carlotti & Sciandra 1989) and *Calanus finmarchicus* in the North Atlantic (Carlotti 1996, Carlotti & Radach 1996). These utilise parameters for ingestion, egestion, excretion, reproduction, mortality, growth rate and mass on moulting, hatching and moulting. Ingestion, egestion and excretion rates have been established for the biomass dominant species (Schnack-Schiel et al. 1991, Atkinson et al.1992, Hopkins et al .1993, Pasternak et al. 1994), whilst egg production and egg hatching times in relation to temperature have been established by Ward & Shreeve (1995, 1998). Now with the estimates of mortality, the mass of each copepodite stage upon moulting, and the stage duration of the range of copepodite stages, presented in this thesis and in Shreeve & Ward (1998) and Shreeve et al. (2002), it should be possible to begin to apply population dynamics models to these two key species in the Southern Ocean.

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Appendix 1 Oxygen concentration in filtered sea-water

Introduction

Water which was used to incubate the animals in during the moulting rate experiments was collected from a pumped non-toxic sea water supply from an inlet at 7 m depth in the ships hull. This was subsequently filtered through a series of three Balston ® filters of pore size 25, 1 and 0.2 μm, to remove predators and bacteria. The concentration of oxygen in the sea water post filtration was of concern, as a reduced concentration may stress the copepods and change their physiology, which may effect their moulting rates. Oxygen concentration in the sea water both pre- and post-filtration were therefore analysed in controlled facilities in the laboratories in Cambridge using a technique developed by Peck and Uglow (1990) and improved by Peck and Whitehouse (1992). Peck and Whitehouse (1992) designed a desorber for couloximetric determinations of oxygen in sea water which decreased the lower limit of oxygen detection to around 0.13 pg of oxygen.

Material and Method

Sea water was collected from the North Sea at Lowestoft beach and filtered through a Cansdale filter which is routinely used at the British Antarctic Survey HQ to maintain Antarctic marine life in aquaria. Samples of this water were used to estimate the concentration of dissolved oxygen in the sea water both prior to and after filtration. A 50 litre sample of sea water was aerated and maintained at 2.5 °C in a constant temperature room for 12 hours, this temperature was similar to ambient sea-surface water

temperatures recorded around island of South Georgia.

A sub-sample of 25µl of this water was then taken and injected into the Couloximeter (Peck & Uglow 1990), this was repeated until two samples produced a value for the dissolved oxygen concentration with less than 2% variation, this was the 'control'. The rest of the 50 l sample was then filtered through a series of three Balston ® filters of pore size 25, 1 and 0.2 µm. This process raised the temperature of the sea water by 0.5 °C to 3 °C. Sub-samples were removed and tested as before for dissolved oxygen concentration both immediately after filtration and again after 5 hours when the temperature had settled back to 2.5 °C.

Results & Discussion

Mean dissolved oxygen concentration of the control was 316 µmol O_2 Kg, equivalent to 95 % saturation at that temperature. Immediately after filtration the temperature had risen by 0.5 °C, and oxygen concentration had increased to 326 µmol O_2 Kg (99 % saturation). After leaving the sample for 5 hours to return to 2.5 °C, a mean value of 321 µmol O_2 Kg (97 % saturation) was measured. A one way ANOVA showed no significant difference between the dissolved oxygen concentration of the pre- and post- filtered sea water ($n_1 = 2$, $n_2 = 2$, $F = 3.31$, $p > 0.2$). As the oxygen concentration was not reduced by this method of filtration but temperature was raised slightly we proceeded to filter and chill incubation water to ambient sea water surface temperature prior to use in the incubation experiments.

Appendix II

Calculation of the stage duration of copepodite stage I *Calanoides acutus*.

Calanoides acutus stage CI was never found in sufficient numbers in the plankton samples taken around South Georgia to facilitate their incubation in moulting rate studies. However a study in the Scotia Sea during austral summer 2000 provided an opportunity to estimate the stage duration of CI *C. acutus*.

Individuals used in moulting rate experiments were sorted from plankton hauls taken at two stations, at 59.91 °S 36.64 °W and 63.23 °S 52.46 °W. Methods for collection, sorting and incubation of the individuals followed the same procedures as set out in chapters 3 and 4. 200 individuals were incubated in total and gave a mean stage duration of 6.4 days. Mean water temperature in the top 60 m from the stations at which these individuals were sorted was however much lower than that experienced during the survey around South Georgia, 0.63 °C compared to 2.18 °C. An estimate of stage duration at the water temperatures that copepodites would have experienced around South Georgia was made by applying Bělehrádek's function as in Chapter 4 (p 96). This gave an estimate of the stage duration at 2.18 °C as being 5.7 days. This has been included in the data in chapter 4, as it is the only estimate available of the duration of stage CI *C. acutus*, and completes the full range of copepodite stage durations.

Rhincalanus gigas. Copepodite I stage duration in relation to each station and year, number of individuals used in each analysis in parenthesis. E and W, eastern and western mesoscale boxes, 1 - 8 refer to station numbers, see Chapter 3, Fig. 3.2 for details. - data not collected.

Station	1995/96	1996/97	1997/98	1998/99
E1	-	-	-	-
E2	-	-	-	-
E3	-	21 (42)	-	-
E4	-	-	-	-
E5	-	-	-	-
E6	-	-	-	8 (52)
E7	-	5(41)	6 (101)	-
E8	-	-	-	-
W1	-	-	21 (31)	17 (58)
W2	-	16 (93)	-	-
W3	-	6 (102)	-	11 (56)
W4	-	-	-	14 (72)
W5	-	-	-	-
W6	-	11 (90)	18 (38)	17 (34)
W7	-	13 (200)	-	44 (66)
W8	-	-	-	17 (61)

Rhincalanus gigas. Copepodite II stage duration in relation to each station and year, number of individuals used in each analysis in parenthesis. E and W, eastern and western mesoscale boxes, 1 - 8 refer to station numbers, see Chapter 3, Fig. 3.2 for details. - data not collected.

Station	1995/96	1996/97	1997/98	1998/99
E1	-	19 (57)	-	-
E2	-	15 (82)	-	-
E3	-	49 (74)	-	27 (122)
E4	-	12 (56)	-	4 (49)
E5	-	-	-	-
E6	-	-	-	31 (169)
E7	-	97 (97)	12 (121)	-
E8	-	-	-	11 (34)
W1	-	-	18 (63)	-
W2	-	39 (97)	8 (111)	-
W3	-	53 (133)	13 (107)	7 (47)
W4	-	-	19 (76)	18 (35)
W5	-	-	-	-
W6	-	31 (108)	25 (38)	10 (52)
W7	-	31 (110)	11 (50)	-
W8	-	-	-	11 (32)

Rhincalanus gigas. Copepodite III stage duration in relation to each station and year, number of individuals used in each analysis in parenthesis. E and W, eastern and western mesoscale boxes, 1 - 8 refer to station numbers, see Chapter 3, Fig. 3.2 for details. - data not collected.

Station	1995/96	1996/97	1997/98	1998/99
E1	-	-	-	-
E2	-	-	-	-
E3	-	-	-	-
E4	-	-	-	43 (107)
E5	-	-	-	-
E6	-	-	-	-
E7	-	-	-	-
E8	-	-	-	32 (48)
W1	-	-	56 (56)	37 (37)
W2	-	12 (35)	49 (98)	-
W3	-	13 (58)	116 (58)	37 (129)
W4	-	-	-	20 (39)
W5	-	-	25 (63)	-
W6	-	12 (36)	-	-
W7	-	21 (85)	-	15 (30)
W8	-	-	-	7 (39)

Calanoides acutus . Copepodite II stage duration in relation to each station and year, number of individuals used in each analysis in parenthesis. E and W, eastern and western mesoscale boxes, 1 - 8 refer to station numbers, see Chapter 3, Fig. 3.2 for details. - data not collected.

Station	1995/96	1996/97	1997/98	1998/99
E1	-	-	-	-
E2	-	-	-	-
E3	-	-	-	-
E4	-	-	-	-
E5	-	-	-	-
E6	-	-	-	-
E7	-	-	-	-
E8	-	-	-	-
W1	-	-	-	-
W2	-	-	-	-
W3	-	-	-	8 (61)
W4	-	-	-	6 (56)
W5	-	-	-	
W6	-	6 (38)	-	4 (55)
W7	-	4 (66)	-	3 (128)
W8	-	-	-	6 (34)

Calanoides acutus. Copepodite III stage duration in relation to each station and year, number of individuals used in each analysis in parenthesis. E and W, eastern and western mesoscale boxes, 1 - 8 refer to station numbers, see Chapter 3, Fig. 3.2 for details. - data not collected.

Station	1995/96	1996/97	1997/98	1998/99
E1	-	-	-	-
E2	-	-	4 (33)	-
E3	-	10 (48)	-	-
E4	-	-	-	11 (54)
E5	-	-	-	5 (126)
E6	-	-	6 (54)	6 (44)
E7	-	-	7 (90)	5 (64)
E8	-	-	-	7 (89)
W1	-	-	22 (88)	6 (82)
W2	-	7 (124)	-	-
W3	14 (42)	5 (88)	5 (90)	8 (125)
W4	-	-	15 (106)	8 (133)
W5	-	-	6 (64)	-
W6	8 (48)	16 (112)	-	-
W7	-	9 (99)	-	8 (30)
W8	-	-	-	8 (87)

Calanoides acutus. Copepodite IV stage duration in relation to each station and year, number of individuals used in each analysis in parenthesis. E and W, eastern and western mesoscale boxes, 1 - 8 refer to station numbers, see Chapter 3, Fig. 3.2 for details. - data not collected.

Station	1995/96	1996/97	1997/98	1998/99
E1	-	-	-	-
E2	-	12 (69)	12 (111)	-
E3	-	16 (86)	-	10 (54)
E4	23 (69)	6 (59)	-	9 (70)
E5	-	20 (30)	56 (115)	6 (83)
E6	-	160 (80)	116 (174)	21 (114)
E7	-	34 (86)	10 (144)	-
E8	-	49 (123)	37 (147)	7 (86)
W1	-	-	26 (208)	8 (127)
W2	-	11 (55)	9 (276)	10 (31)
W3	13 (98)	13 (57)	7 (186)	10 (80)
W4	-	-	23 (256)	8 (146)
W5	-	-	16 (180)	-
W6	16 (64)	13 (53)	82 (164)	15 (30)
W7	-	8 (60)	-	-
W8	-	-	55 (218)	-